DK04/529

Kongeriget Danmark

Patent application No.: PA 2004 00570

Date of filing: 07 April 2004

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Titlel: Use of a ghrelin-like compound

IPC: -

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17 August 2004

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PATENT- OG VAREMÆRKESTYRELSEN

Use of a ghrelin-like compound

All patent and non-patent references cited in the application, or in the present application, are also hereby incorporated by reference in their entirety.

Field of invention

The present invention relates to the use of a ghrelin-like compound for the production of medicament for the treatment or prevention of cachexia, stimulation of appetite, food intake and/or weight gain as well as to a method of treating or preventing cachexia, stimulating appetite, food intake and/or weight gain in an individual in need thereof by administering a ghrelin-like compound. Furthermore, the invention relates to ghrelin-like compounds, pharmaceutical compositions comprising ghrelin-like compounds, and medical packagings comprising the pharmaceutical compositions.

Background of invention

Ghrelin is a bioactive peptide which originally was described to be involved in the control of GH secretion but later found to be a major regulator of appetite, food intake and energy homeostasis (1;2). As many other bioactive peptides ghrelin probably act both as a hormone, a paracrine substance and as a neurotransmitter.

The story of ghrelin, its receptor and synthetic compounds acting through this receptor unraveled in a unique "reverse" order. In the eighties a synthetic hexa-peptide from a series of opioid-like peptides was found to be able to release growth hormone (GH) from isolated pituitary cells (3). Since this action was independent of the growth hormone releasing hormone (GHRH) receptor, several pharmaceutical companies embarked upon drug discovery projects based on this hexa-peptide GH secretagogue (GHS) and its putative receptor. Several series of potent and efficient peptide as well as non-peptide GH secretagogues were consequently described in the mid nineties (4-6). However, first several years later was the receptor through which these artificial GH secretagogues acted eventually cloned and shown to be a member of the 7TM G protein coupled receptor family (7;8). But, first in 1999 was the endogenous ligand for this receptor the hormone ghrelin finally discovered (9).

The main site for ghrelin production is the stomach, where the peptide is found in classical endocrine cells in the gastric mucosa.

From here, ghrelin is secreted in the pre-meal situation which results in a sharp, short-lived surge in plasma levels of ghrelin before the meal and starting 1-2 hours before and lasting a short while after initiation of the meal. Since ghrelin is the only peripherally produced orexigenic (appetite promoting) substance it is believed that the increase in plasma levels of ghrelin is crucial for the initiation of the meal.

In its role as a key initiator of appetite, ghrelin released from the endocrine cells in the mucosa of the GI tract may act both locally as a paracrine substance and centrally as a hormone. Locally, ghrelin may act as an initiator of afferent activity in for example afferent vagal neurons. Such neurons will relay the ghrelin stimulus to centers in the CNS such as the nucleus tractus solitarius (NTS) which further communicate with appetite and energy homeostasis regulatory centers such as the paraventricular nucleus and arcuate nucleus in the hypothalamus. As a hormone, ghrelin is believed to act on central appetite regulating POMC and NPY/AGRP neurons, which express ghrelin receptors. Most of these neurones in the arcuate nucleus as such are located inside the blood brain barrier and is consequently not accessible to blood born messengers such as ghrelin. However, some POMC and NPY/AGRP neurons are found in the nearby median eminence a cicumventricular organ, which is clearly outside the blood brain barrier and are therefore target for hormonally transmitted ghrelin signaling from the GI tract. However recently it has been described that ghrelin is transported across the blood brain barrier (10). It is important to note that at the central appetite regulatory center for example at the NPY / AGRP neurons – i.e. the first level neurons in the stimulatory branch of appetite control – ghrelin acting through stimulatory ghrelin receptors is the only stimulatory input known from the periphery. All other hormones and neurotransmitters: leptin, insulin, PYY3-36, a-MSH etc. act as inhibitors on the NPY/AGRP neurons in this important "appetite gate-keeping" center

Previously, ghrelin has been administered by continuous infusions for 270 hours, which has shown that an increase in food intake can be obtained through intra- venous administration of ghrelin. The doses were 5pmol/kg/min, giving in an average test person at 70 lg a total infusion of 3200mg (Wren et al JCEM 2001; 86(12)5992-

5995). Recently, it was shown that infusion of ghrelin for 90 minutes could increase food intake by 30 % in cancer cachexia patients. (Abstract P09 Digestive Hormones, Appetite and Energy Balance, Baylis and Starling meeting, London, June 2003).

These studies demonstrate that parenteral administration of ghrelin can increase appetite in both normal subjects and in patients with loss of appetite. However, a prolonged infusion regimen is clearly not an optimal administration form for both practical reasons and for physiological reasons.

Summary of the Invention

The present invention relates to the use of a ghrelin-like compound, including human ghrelin, in the treatment or stimulation of one or more of the following conditions, or in the manufacture of a medicament for treating or stimulating or *increasing, respectively, one or more of:*

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increasing body fat mass,

including any combination of the above.

Preferred combinations are: a); b); c); d); e); and f) in isolation; as well as a) + c); a) + d); a) + e); a) + f); b) + c); b) + d); b) + e); b) + f); a) + c) + d); a) + c) + e); a) + c) + f); a) + d) + e); a) + d) + f); a) + e) + f); a) + c) + d) + e); a) + c) + d) + f); a) + d) + e) + f); a) + c) + d) + e) + f); b) + c) + d); b) + c) + e); b) + c) + f); b) + d) + e); b) + d) + f); b) + e) + f); b) + c) + d) + e); b) + c) + d) + f); b) + d) + e) + f); and b) + c) + d) + e) + f).

Although prior art references has shown some effect of ghrelin in the treatment of the above conditions, the prior art has only shown this for administration forms that are of little interest when administering a medicament for a prolonged period. Prior

art methods have included intravenous administration and intratechal administration. Due to the short half-life of acylated ghrelin (T½=10 min) no other administration forms have been available to the person skilled in the art when treating the above conditions.

On the contrary with respect to other conditions, such as conditions relating to the heart, wherein it has been shown (Baldanzi et al. "Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT", The Journal of Cell Biology, 1029-1037, Vol 159, 2002) that both the acylated and the des-acylated ghrelin has an effect, however, in relation to conditions wherein an increased food intake and weight gain is desired, only the acylated ghrelin is effective.

The present inventors have found that it is possible to obtain a sufficient effect of ghrelin when administered subcutaneously, in particular when administered subcutaneously prior to a meal, thereby ensuring a close mimic of the natural pre-meal situation.

Accordingly, the present invention relates to the use of a ghrelin-like compound for the preparation of a medicament for

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increasing body fat mass,

including any combination of the above,

in an individual by administering a subcutaneous dosage of said medicament to the individual,

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids.

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

Preferred combinations are: a); b); c); d); e); and f) in isolation; as well as a) + c); a) + d); a) + e); a) + f); b) + c); b) + d); b) + e); b) + f); a) + c) + d); a) + c) + e); a) + c) + f); a) + d) + e); a) + d) + f); a) + e) + f); a) + c) + d) + e); a) + c) + d) + f); a) + d) + e) + f); a) + c) + d) + e) + f); b) + c) + d); b) + c) + e); b) + c) + f); b) + d) + e); b) + d) + f); b) + e) + f); b) + c) + d) + e); b) + c) + d) + f); b) + d) + e) + f); and b) + c) + d) + e) + f).

In another aspect, the invention relates to the use of a ghrelin-like compound for the preparation of a medicament for

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or

c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increasing body fat mass,

including any combination of the above,

in an individual by administering a dosage of said medicament to the individual prior to or during a meal, said dosage comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin,

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

Preferred combinations are: a); b); c); d); e); and f) in isolation; as well as a) + c); a) + d); a) + e); a) + f); b) + c); b) + d); b) + e); b) + f); a) + c) + d); a) + c) + e); a) + c) + f); a) + d) + e); a) + d) + f); a) + e) + f); a) + c) + d) + e); a) + c) + d) + f); a) + d) + e) + f); a) + c) + d) + e) + f); b) + c) + d); b) + c) + e); b) + c) + f); b) + d) + e); b) + d) + f); b) + e) + f); b) + c) + d) + e); b) + c) + d) + f); b) + d) + e) + f); and b) + c) + d) + e) + f).

The medicament can be administered as a bolus injection or by fast running infusion, i.e. an infusion preferably lasting less than 120 minutes, such as less than 90 minutes, for example less than 60 minutes, such as less than 45 minutes, such as less than 30 minutes, for example less than 25 minutes, such as less than 20 minutes, such as less than 15 minutes, for example less than 12 minutes, such as less than 10 minutes, such as less than 8 minutes, for example less than 6 minutes, such as less than 5 minutes, such as less than 4 minutes, for example less than 3 minutes, such as less than 2 minutes, such as less than 1 minute.

A Y-formed catheter can be used for rapid infusion. A solution of the ghrelin-like compound can be injected through one catheter entry port and optionally saline can, if desirable, be injected through the other catheter entry port.

The bolus injection or the fast running infusion can be administered prior to a meal or during a meal as described in more detail herein below. In one preferred embodiment the medicament is administered as a bolus. The bolus is can preferably be administered subcutaneously.

Also, the invention relates to novel compounds relevant for the therapeutic and prophylactic indications herein. Accordingly, in another aspect the invention relates to a ghrelin-like compound wherein the ghrelin-like compound is defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic amino acids, said amino acid being modified with a acylgroup, wherein the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

Z^2 is an optionally present protecting group,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

m is 0 or an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

Furthermore, the invention relates to a pharmaceutical composition comprising a ghrelin-like compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients as well a use of the compounds for the preparation of a medicament.

In a preferred aspect of the invention the ghrelin-like compound is administered with a substance capable of increasing the half-life of the ghrelin-like compound, for example by incorporating the ghrelin-like compound into liposomes, micelles, iscoms, and/or microspheres or other transport molecules, in particular to protect the modified amino acid from being desacylated. Accordingly, the invention further relates to a pharmaceutical composition comprising the compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients said composition further comprising transport molecules, such as liposomes, micelles, and/or microspheres.

In yet a further aspect the invention relates to a medical packaging comprising one or more dosage units of a pharmaceutical composition comprising a compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients.

In a preferred embodiment the medical packaging comprises the pharmaceutical composition as defined above with predefined amounts of dosage units.

In one aspect the invention relates to a medical packaging comprising a pharmaceutical composition comprising a compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients, said packaging having from one to three dosage units.

In another aspect the invention relates to a medical packaging comprising a pharmaceutical composition comprising a compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients, said packaging having from 7 to 28 dosage units.

The invention further relates to a method for the

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increase of body fat mass,

including any combination thereof,

in an individual by administering subcutaneously an effective dosage of a medicament comprising a ghrelin-like compound to said individual,

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1-(X^1)_m-(X^2)-(X^3)_n-Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

Preferred combinations are: a); b); c); d); e); and f) in isolation; as well as a) + c); a) + d); a) + e); a) + f); b) + c); b) + d); b) + e); b) + f); a) + c) + d); a) + c) + e); a) + c) + f); a) + d) + e); a) + d) + f); a) + e) + f); a) + c) + d) + e); a) + c) + d) + f); a) + d) + e) + f); a) + c) + d) + e) + f); b) + c) + d); b) + c) + e); b) + c) + f); b) + d) + e); b) + d) + f); b) + e) + f); b) + c) + d) + e); b) + c) + d) + f); b) + d) + e) + f); and b) + c) + d) + e) + f).

In yet another aspect the invention relates to a method for the
a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or

f) increase of body fat mass,

including any combination thereof,

in an individual by administering an effective dosage of a medicament comprising a ghrelin-like compound to said individual prior to a meal,

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

Preferred combinations are: a); b); c); d); e); and f) in isolation; as well as a) + c); a) + d); a) + e); a) + f); b) + c); b) + d); b) + e); b) + f); a) + c) + d); a) + c) + e); a) + c) + f); a) + d) + e); a) + d) + f); a) + e) + f); a) + c) + d) + e); a) + c) + d) + f); a) + d) + e)

+ f); a) + c) + d) + e) + f); b) + c) + d); b) + c) + e); b) + c) + f); b) + d) + e); b) + d) + f); b) + e) + f); b) + c) + d) + e); b) + c) + d) + f); b) + d) + e) + f); and b) + c) + d) + e) + f).

Detailed Description of the Invention

Definitions

Affinity: the strength of binding between receptors and their ligands, for example between an antibody and its antigen.

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, the amino acid encompasses every amino acid such as L-amino acid, D-amino acid, alpha -amino acid, beta -amino acid, gamma -amino acid, natural amino acid and synthetic amino acid or the like as long as the desired functional property is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide, abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		
1-Letter	3-Letter	AMINO ACID
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine

P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
Z	Glx	Glu and/or Gln
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
B	Asx	Asn and/or Asp
C	Cys	cysteine
X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences represented herein by formulae have a left-to-right orientation in the conventional direction of amino terminus to carboxy terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and non-naturally occurring amino acids. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or a covalent bond to an amino-terminal group such as NH_2 or acetyl or to a carboxy-terminal group such as COOH.

Appetite: Appetite in an individual is assessed by measuring the amount of food ingested and by assessing the individual's desire to eat. Appetite (i.e., hunger) is typically assessed with a short questionnaire given to individuals on a random basis several times a week. Typically, subjects rate their hunger, preoccupation with food, and desire to eat greater quantities and different types of food by answering the questions using analogue scales ranging from 1, not at all, to 5, extremely.

BMI measures your height/weight ratio. It is determined by calculating weight in kilograms divided by the square of height in meters. The BMI"normal" range is 19-22.

Body fat mass: Body fat mass can be measured e.g. by the fat fold technique: In this technique, a pincer-type caliper is used to measure subcutaneous fat by determining skin fold thickness at representative sites on the body. These skin fold measurements are then used to compute body fat by either adding the scores from the various measurements and using this value as an indication of the relative degree of fatness among individuals or by using the measurements in mathematical equations that have been developed to predict percent body fat.

Concentration equivalent: A concentration equivalent is an Equivalents dosage being defined as the dosage of a ghrelin-like compound having in vitro and/or in vivo the same response as evaluated from a dosage-response curve as wild-type ghrelin.

Dissociation constant, Kd: a measure to describe the strength of binding (or affinity or avidity) between receptors and their ligands, for example an antibody and its antigen.The smaller Kd the stronger binding.

Fusion Polypeptide: A polypeptide comprised of at least two polypeptides and a linking sequence to operatively link the two polypeptides into one continuous polypeptide. The two polypeptides linked in a fusion polypeptide are typically derived from two independent sources, and therefore a fusion polypeptide comprises two linked polypeptides not normally found linked in nature.

Ghrelin: a polypeptide as described in Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656-660. Human 28 aa ghrelin has the amino acid of SEQ ID NO: 1.

GHS: growth hormone secretagogue

GHS-R 1a: the receptor for GHS. GHS-R 1a is also denoted GHS 1a.

Immunologically distinct: The phrase immunologically distinct refers to the ability to distinguish between two polypeptides on the ability of an antibody to specifically bind one of the polypeptides and not specifically bind the other polypeptide.

Individual: A living animal or human in need of susceptible to a condition, in particu-

lar a cachectic condition as defined herein. In preferred embodiments, the subject is a mammal, including humans and non-human mammals such as dogs, cats, pigs, cows, sheep, goats, horses, rats, and mice. In the most preferred embodiment, the subject is a human.

Isolated: is used to describe the various ghrelin-like compounds, polypeptides and nucleotides disclosed herein, that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified.

Modified amino acid: an amino acid wherein an arbitrary group thereof is chemically modified. In particular, a modified amino acid chemically modified at the alpha - carbon atom in an alpha -amino acid is preferable.

Monoclonal Antibody: The phrase monoclonal antibody in its various grammatical forms refers to a population of antibody molecules that contains only one species of antibody combining site capable of immunoreacting with a particular antigen.

Multimeric: A polypeptide molecule comprising more than one polypeptide. A multimer may be dimeric and contain two polypeptides and a multimer may be trimeric and contain three polypeptides. Multimers may be homomeric and contain two or more identical polypeptides or a multimer may be heteromeric and contain two or more nonidentical polypeptides.

Polyclonal antibody: Polyclonal antibodies is a mixture of antibody molecules recognising a specific given antigen, hence polyclonal antibodies may recognise different epitopes within said antigen.

Polypeptide: The phrase polypeptide refers to a molecule comprising amino acid residues which do not contain linkages other than amide linkages between adjacent amino acid residues.

Receptor: A receptor is a molecule, such as a protein, glycoprotein and the like, that can specifically (non-randomly) bind to another molecule.

Secretagogue: a growth hormone secretagogue, ie. a substance stimulating growth hormone release, such as ghrelin or a ghrelin-like compound.

Specificity: The term specificity refers to the number of potential antigen binding sites which immunoreact with (specifically bind to) a given polypeptide. The polypeptide may be a single polypeptide or may be two or more polypeptides joined by disulfide bonding.

Indications

The present invention relates to the use of a ghrelin-like compound in the treatment or prophylaxis of conditions e.g. relating to pathological weight or fat loss, including

a) prophylaxis or treatment of cachexia, and/or
b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increase of body fat mass,

Cachexia

The word cachexia comes from the Greek kakos for "bad" and hexis for "condition." Cachexia is one of the most distressing and devastating symptoms of several severe diseases, such as cancer, robbing people of their energy, sense of well-being, and quality of life, and increasing their dependence on others. Cachexia often accompanies malignancies of the pancreas, stomach, esophagus, lung, and intestines.

The foremost sign of cachexia is weight loss, not only of fatty tissue but also of muscle tissue and even bone. This non-fatty tissue is also known as "lean body mass."

In addition, there is loss of appetite (anorexia), weakness (asthenia), and a drop in hemoglobin level (anemia).

Treatment of cachexia is not simply a matter of eating more. Even if the person wants to eat, even if he or she tries to eat, even if the person is given nutrients through a stomach tube or intravenously, the condition will normally not be reversed.

Recent research has revealed that the condition is now regarded as part of the body's reaction to the presence of the underlying disease. Recent research also indicates that, in some cases, tumors themselves produce substances that induce cachexia.

Cachexia, or wasting, as it may also be called is seen with several diseases, such as AIDS, cancer, chronic heart failure, chronic lung disease, such as COLD, COPD, liver cirrhosis, renal failure, and autoimmune diseases such as rheumatoid arthritis and systemic lupus, sepsis and severe infection. Furthermore, wasting is also seen in aging.

Lipodystrophy

Lipodystrophic syndromes encompass a heterogeneous group of rare disorders characterized by partial or generalized loss of adipose tissue depots [Am J Med 2000 108, 143-152]. There are several different types of lipodystrophies and the degree of fat loss may vary from very small depressed areas to near complete absence of adipose tissue Some patients may have only cosmetic problems while others may also have severe metabolic complications such as dyslipidemia, hepatic steatosis, and severe insulin resistance [Trend Endo Meta 200011:410-416]. These disorders can either be inherited (familial or genetic lipodystrophies) or can occur secondary to various types of illnesses or drugs (acquired lipodystrophies).

Stimulation of appetite, food intake, weight gain, increase of body fat mass

As written above, facilitating a weight gain and facilitating maintenance of weight, in particular in individuals suffering from a pathologically weight loss, is not only a matter of stimulating appetite and/or food intake.

Accordingly, in one aspect the present invention relates to the stimulation of appetite by administering a ghrelin-like compound. Stimulation of appetite, does not necessarily lead to an increase in food intake, and accordingly, the present invention further relates to in another aspect:

the stimulation of food intake by administering a ghrelin-like compound.

And in a third aspect stimulation of weight gain by administering a ghrelin-like compound.

Preferably the ghrelin-like compound is useful for stimulating food intake and weight gain, more preferably the ghrelin-like compound is useful for stimulating weight gain.

As discussed below it is preferred that the ghrelin-like compound is administered prior to a meal, such as within 45 minutes of a meal. Furthermore, it is preferred that the ghrelin-like compound is administered subcutaneously.

Furthermore, the ghrelin-like compounds may be administered to facilitate maintenance of physical functioning, and/or facilitate recovery of physical function, for example in individuals recovering from major surgeries, such as insertion of a hip prosthesis, amputations, and bone fractures.

In particular the present invention is useful for treatment of under weight subjects, or for preventing loss of weight to a stage of under weight. Under weight subjects include those having a body weight about 10% or less, 20% or less, or 30% or less, than the lower end of "normal" weight range or Body Mass Index ("BMI")."Normal" weight ranges are well known in the art and take into account factors such as a patient age, height, and body type.

Increasing weight or appetite can be useful for maintaining weight or producing a weight or appetite gain in an under weight subject, or in a patient having a disease or undergoing treatment that affects weight or appetite. In addition, for example, farm animals such as pigs, cows and chickens can be treated to gain weight.

An increase in the body fat mass of an individual can be readily assess by the skilled person using a number of state of the art techniques. In one embodiment the invention relates to an increase in body fat mass without the individual gaining weight overall.

Subcutaneous administration

It is important to note that ghrelin receptors are found in a number of places in the organism besides on the GH producing cells and in the hypothalamic centers for appetite etc. control. It the CNS these receptors are tuned to receiving signals from local ghrelin containing neurons. Peripherally secreted or artificially administered ghrelin will not reach such sites due to the blood brain barrier, however currently available so-called GH secretagouges, which are small organic compounds such as MK-0677, will pass the blood brain barrier and also reach these sites – and consequently have the danger of causing unwanted side effects. Thus such compounds which do have the advantage of being for example orally active will not be optimal for mimicking the natural pre-meal, appetite inducing surge of ghrelin, since they will reach basically all ghrelin receptors in the body. In contrast, by using the natural peptide, ghrelin itself or homologues thereof, and administering it peripherally – as in the present invention – it is ensured that only the relevant, appetite regulating ghrelin receptors are reached and stimulated.

Any parenteral administration form that will ensure that the ghrelin receptors which normally are the target for peripherally produced ghrelin in the premeal situation will be exposed to sufficient levels of the bioactive form of ghrelin to ensure robust and *appropriate appetite stimulation without causing desensitization of the system may* be part of the present invention. However, taken into consideration that the individuals to be treated possibly will have to receive treatment for a longer period, such as weeks or months, it is preferred that the administration form is well suited herefor.

Accordingly, it is preferred that the ghrelin-like compound according to the invention is administered subcutaneously in an amount sufficient to allow sufficient levels of the bioactive form of ghrelin, i.e. the acylated form, to reach the receptors in time, such as prior to the forthcoming meal.

The present invention preferably deals with methods for administering ghrelin in a way which mimics the physiologically pre-meal situation as closely as possible yet providing patients in need of increased food intake, for example fragile elderly, post operative patients, patients with lost appetite as part of cachexia for example precipitated by cancer, cardiac disease etc. with a sufficient extra stimulatory input to their appetite regulating ghrelin receptors, which normally are reached by ghrelin in the pre-meal situation.

Bolus administration

Furthermore, from a molecular pharmacological point-of-view it is important to note that it has been found that the ghrelin receptor normally is exposed to short-lived surges in the concentrations of the natural agonist ligand, ghrelin. The GHS-R 1a receptor belongs to the class of receptors, so-called G protein coupled receptors or 7TM receptors, that upon continued exposure to an agonist will be desentizised, internalized and down-regulated. These mechanisms, which are inherent to the overall signal transduction system, involve processes such as receptor phosphorylation (which in itself decreases the affinity of the receptor for the agonist) binding of inhibitory proteins such as arrestin (which sterically block the binding of signal transduction molecules such as G proteins). Other part of the agonist mediated desensitization process is receptor internalization (i.e. physical removal of the receptor from the cell surface where it could bind the agonist) as well as receptor down regulation (i.e. decreased production / expression of the receptor). Receptor internalization could after short-lived exposure of the receptor to agonist be followed by a resensitization process, where the receptor is dephosphorylated and recycled to the cell surface to be used again. Without being bound by theory, it is believed that, upon prolonged stimulation, which would occur for example during a long-lasting continuous infusion of the agonist, the receptor down-regulation process ensures that the target cell is adjusted in its signal transduction system etc. to this situation.

The present invention provides a procedure for an optimal administration of ghrelin to patients in order to obtain a maximal response and avoid for example desensitization mechanisms.

Accordingly, the present invention relates in one aspect to administration of the ghrelin-like compound in boluses, preferably a bolus prior to each main meal. It has been found, in contrary to the prolonged administration processes in the prior art, that a bolus administration leads to not only stimulation of appetite, but also to stimulation of feed intake and more important to stimulation of weight gain.

Without being bound by theory, it is believed that premeal subcutaneous injection, intravenous injection or short-term infusions of appropriate doses of ghrelin will ensure that a robust stimulation of appetite inducing ghrelin receptors will be obtained with minimal constraint to the mobility etc. of the patient. Thus for example patients with hip fractures can in the post operative situation be treated in the premeal period and if required during the meal as such, but will be free to move around and participate in the important post operative physicotherapeutic regimens.

Ghrelin-like compound

A ghrelin-like compound according to the invention described herein is a compound comprising a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

Accordingly, the term "ghrelin-like compound" includes the naturally occurring 28 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 1, as well as the naturally occurring 27 aa human ghrelin, the amino acid of which is shown in SEQ ID NO: 2. Thus, the present invention relates to the use of ghrelin or a peptide homologous thereto. Ghrelin is described by Kojima in Nature (1999), vol. 402,656-660.

The present invention includes diastereomers as well as their racemic and resolved enantiomerically pure forms. Ghrelin-like compounds can contain D-amino acids, L-amino acids, alpha-amino acid, beta-amino acid, gamma-amino acid, natural amino acid and synthetic amino acid or the like or a combination thereof. Preferably, amino acids present in a ghrelin-like compound are the L-enantiomer.

The ghrelin-like compound comprises an amino acid modified with a bulky hydrophobic group. The number of amino acids N-terminally to the modified amino acid is preferably within the range of from 1-9. Accordingly, m is preferably an integer in the range of from 1-9, such as of from 1-8, such as of from 1-7, such as of from 1-6, such as of from 1-5, such as of from 1-4, such as of from 1-3, such as of from 1-2, such as 2.

It is more preferred that the number of amino acids N-terminally to the modified amino acid is low, such as of from 1-3, such as of from 1-2. Most preferably 2 amino acids are positioned N-terminal to the modified amino acid.

In a preferred embodiment $(X^1)_m$ has a Gly residue in the N-terminal part of the sequence. Accordingly, in preferred embodiment $(X^1)_m$ is selected from the sequences:

Gly, Gly-Ser, Gly-Cys, Gly-Lys, Gly-Asp, Gly-Glu, Gly-Arg, Gly-His, Gly-Asn, Gly-Gln, Gly-Thr, and Gly-Tyr.

More preferably $(X^1)_m$ is selected from , Gly-Ser, and Gly-Cys, most preferably from Gly-Ser.

In another words, in a preferred embodiment the ghrelin-like compound is selected from a compound of

formula II Z^1 – Gly- $(X^1)_{m-1}$ – (X^2) – $(X^3)_n$- Z^2,

formula III Z^1 – Gly- Ser – (X^2) – $(X^3)_n$- Z^2, and

formula IV Z^1 – Gly – (X^2) – $(X^3)_n$- Z^2.

And more preferably the ghrelin-like compound has formula III.

As described above, X^2 may be any amino acid modified with a bulky hydrophobic group. In particular X^2 is selected from the group of modified Ser, Cys, Asp, Lys, Trp, Phe, Ile, and Leu. More preferably X^2 is selected from the group of modified Ser, modified Cys and modified Lys, and most preferably X^2 is modified Ser.

Furthermore, $(X^1)_m$ – (X^2) is preferably Gly-Xaa-Ser*, or Gly-Xaa-Cys*, wherein Xaa is any amino acid, more preferably $(X^1)_m$ – (X^2) is Gly-Ser-Ser*, or Gly-Ser-Cys*, wherein * indicates that the amino acid residue is modified with a bulky hydrophobic group.

$(X^3)_n$ preferably comprises a sequences which is a fragment of ghrelin, or a variant of a fragment of ghrelin, such as human ghrelin. Accordingly, $(X^3)_n$ preferably comprises a sequence selected from one or more of the sequences shown below:

Phe Leu Ser Pro Glu His Gln
Phe Leu Ser Pro Glu His
Phe Leu Ser Pro Glu
Phe Leu Ser Pro

Phe Leu Ser
Phe Leu
Phe

In a preferred embodiment the length of the ghrelin-like compound is substantially similar to the length of human ghrelin, i.e. 27 or 28 amino acids. Accordingly, n is preferably an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, or such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24.

$(X^3)_n$ may be selected from any fragment of ghrelin, such as human ghrelin, and accordingly, $(X^3)_n$ may be selected from one or more of the sequences shown below or a homologue thereof:

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg
Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln
Phe Leu Ser Pro Glu His Gln Arg Val
Phe Leu Ser Pro Glu His Gln Arg
Phe Leu Ser Pro Glu His Gln
Phe Leu Ser Pro Glu His
Phe Leu Ser Pro Glu
Phe Leu Ser Pro
Phe Leu Ser
Phe Leu
Phe

Or selected from

Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys Pro
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys Lys
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser Lys
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu Ser
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys Glu
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg Lys
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln Arg
Phe Leu Ser Pro Glu His Gln Lys Val Gln Gln
Phe Leu Ser Pro Glu His Gln Lys Val Gln

Phe Leu Ser Pro Glu His Gln Lys Val
Phe Leu Ser Pro Glu His Gln Lys

Or selected from

Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys Lys
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser Lys
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu Ser
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys Glu
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg Lys
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln Arg
Phe Leu Ser Pro Glu His Gln Arg Ala Gln Gln
Phe Leu Ser Pro Glu His Gln Arg Ala Gln
Phe Leu Ser Pro Glu His Gln Arg Ala

Or selected from

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys Pro

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu Ser

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys Glu

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln

Phe Leu Ser Pro Glu His Gln Lys Ala Gln

Phe Leu Ser Pro Glu His Gln Lys Ala

In another embodiment $(X^3)_n$ comprises or consists of a sequence selected from the sequences

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

Functionality

The ghrelin-like compound described herein are active at the GHS receptor. The compounds can bind to the receptor, and preferably, stimulate receptor activity.

GHS receptor activity can be measured using different techniques such as detecting a change in the intracellular conformation of the GHS receptor, in the G-protein coupled activities, and/or in the intracellular messengers.

One simple measure of the ability of a ghrelin like compound to activate the ghrelin receptor is to measure its EC50, i.e. the dose at which the compound is able to activates the signalling of the receptor to half of the maximal effect of the compound. The ghrelin receptor can either be expressed endogenously on primary cells cultures, for example pituitary cells, or heterologously expressed on cells transfected with the ghrelin receptor. Whole cell assays or assays using membranes prepared form either of these cell types can be used depending on the type of assay.

As the ghrelin receptor is generally believed to be primarily coupled to the Gq signalling pathway, any suitable assay which monitor activity in the Gq/G11 signalling pathway can be used, for example:

1) an assay measuring the activation of Gq / G11 performed for example by measurement of GTPgS binding combined with, e.g., anti-Gα–q or -11 antibody precipitation in order to increase the signal to noise ratio. This assay may also detect coupling to other G-proteins than Gq/11.

2) An assay which measure the activity of phopholipase C (PLC) one of the first down-stream effector molecules in the pathway, for example by measuring the accumulation of inositol phosphate which is one of the products of PLC.

3) More down stream in the signalling cascade is the mobilization of calcium from the intracellular stores

4) Further more down stream signalling molecules such as the activity of different kinds of MAP kinases (p38, jun, ect.), NF-κ-B translocation and CRE driven gene transcription may also be measured.

Alternatively binding of fluorescently tagged arrestin to the activated ghrelin receptor may also be used.

In one embodiment the binding of a compound to the receptor GHS-R 1A can be measured by the use of the assay described herein above.

A ghrelin-like compound according to the invention preferably has at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%, functional activity relative to 28 aa human ghrelin as determined using the assay described herein above, and/or an EC50 greater than about 1,000, greater than about 100, or greater than about 50, or greater than about 10. Greater refers to potency and thus indicates a lesser amount is needed to achieve binding inhibition.

In one embodiment of the invention the compound has a potency (EC50) on the GHS-R 1A of less than 500 nM. In another embodiment the compound has a potency (EC50) on the GHS-R 1A of less than 100 nM, such as less than 80 nM, for example less than 60 nM, such as less than 40 nM, for example less than 20 nM, such as less than 10 nM, for example less than 5 nM, such as less than 1 nM, for example less than 0.5 nM, such as less than 0.1 nM, for example less than 0.05 nM, such as less than 0.01 nM.

In a further embodiment the dissociation constant (Kd) of the compound is less than 500 nM. In a still further embodiment the dissociation constant (Kd) of the ligand is less than 100 nM, such as less than 80 nM, for example less than 60 nM, such as less than 40 nM, for example less than 20 nM, such as less than 10 nM, for example less than 5 nM, such as less than 1 nM, for example less than 0.5 nM, such as less than 0.1 nM, for example less than 0.05 nM, such as less than 0.01 nM.

Binding assays can be performed using recombinantly produced GHS receptor polypeptides present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the GHS receptor polypeptide expressed from recombinant nucleic acid or naturally occurring nucleic acid; and also include, for example, the use of a purified GHS receptor polypeptide produced by recombinant means or from naturally occurring nucleic acid which is introduced into a different environment.

Using a recombinantly expressed GHS receptor offers several advantages such as the ability to express the receptor in a defined cell system so that a response to a compound at the GHS receptor can more readily be differentiated from responses at other receptors. For example, the GHS receptor can be expressed in a cell line such as HEK 293, COS 7, and CHO not normally expressing the receptor by an expression vector, wherein the same cell line without the expression vector can act as a control.

Identity and homology

The term "identity" or "homology" shall be construed to mean the percentage of amino acid residues in the candidate sequence that are identical with the residue of a corresponding sequence to which it is compared, after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent identity for the entire sequence, and not considering any conservative substitutions as part of the sequence identity. Neither N- or C-terminal extensions nor insertions shall be construed as reducing identity or homology. Methods and computer programs for the alignment are well known in the art. Sequence identity may be measured using sequence analysis software (e.g., Sequence Analysis Software Package, Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Ave., Madison, Wis. 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications.

A homologue of one or more of the sequences specified herein may vary in one or more amino acids as compared to the sequences defined, but is capable of performing the same function, i.e. a homologue may be envisaged as a functional equivalent of a predetermined sequence.

As described above a homologue of any of the predetermined sequences herein may be defined as:

i) homologues comprising an amino acid sequence capable of being recognised by an antibody, said antibody also recognising the 28 aa human ghrelin, preferably the acylated 28 aa human ghrelin, and/or

ii) homologues comprising an amino acid sequence capable of binding selectively to GHS-R 1a, and/or

iii) homolouges having a substantially similar or higher binding affinity to GHS-R 1a than the 28 aa human ghrelin, preferably the acylated 28 aa human ghrelin.

In the above examples, the 28 aa human ghrelin has the sequence shown in SEQ ID NO: 1, and when acylated is acylated in position 3.

The antibodies used herein may be antibodies binding the N-terminal part of ghrelin or the C-terminal part of ghrelin, preferably the N-terminal part of ghrelin. The antibodies may be antibodies as described in Ariyasu et al. "Delayed short-term secretory regulation of ghrelin in obese animals: Evidensed by a specific RIA for the active form of ghrelin, Endocrinology 143(9):3341-3350, 2002.

Examples of homologues comprises one or more conservative amino acid substitutions including one or more conservative amino acid substitutions within the same group of predetermined amino acids, or a plurality of conservative amino acid substitutions, wherein each conservative substitution is generated by substitution within a different group of predetermined amino acids.

Homologues may thus comprise conservative substitutions independently of one another, wherein at least one glycine (Gly) of said homologue is substituted with an amino acid selected from the group of amino acids consisting of Ala, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one of said alanines (Ala) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Val, Leu, and Ile, and independently thereof, homologues, wherein at least one valine (Val) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Leu, and Ile, and independently thereof, homologues thereof, wherein at least one of said leucines (Leu) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Gly, Ala, Val, and Ile, and independently thereof, homologues thereof, wherein at least one isoleucine (Ile) of said homologues thereof is substituted with an amino acid selected from the group

of amino acids consisting of Gly, Ala, Val and Leu, and independently thereof, homologues thereof wherein at least one of said aspartic acids (Asp) of said homologue thereof is substituted with an amino acid selected from the group of amino acids consisting of Glu, Asn, and Gln, and independently thereof, homologues thereof, wherein at least one of said phenylalanines (Phe) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Tyr, Trp, His, Pro, and preferably selected from the group of amino acids consisting of Tyr and Trp, and independently thereof, homologues thereof, wherein at least one of said tyrosines (Tyr) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Trp, His, Pro, preferably an amino acid selected from the group of amino acids consisting of Phe and Trp, and independently thereof, homologues thereof, wherein at least one of said arginines (Arg) of said fragment is substituted with an amino acid selected from the group of amino acids consisting of Lys and His, and independently thereof, homologues thereof, wherein at least one lysine (Lys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Arg and His, and independently thereof, homologues thereof, wherein at least one of said aspargines (Asn) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Gln, and independently thereof, homologues thereof, wherein at least one glutamine (Gln) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, and Asn, and independently thereof, homologues thereof, wherein at least one proline (Pro) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Phe, Tyr, Trp, and His, and independently thereof, homologues thereof, wherein at least one of said cysteines (Cys) of said homologues thereof is substituted with an amino acid selected from the group of amino acids consisting of Asp, Glu, Lys, Arg, His, Asn, Gln, Ser, Thr, and Tyr.

Conservative substitutions may be introduced in any position of a preferred predetermined sequence. It may however also be desirable to introduce non-conservative substitutions, particularly, but not limited to, a non-conservative substitution in any one or more positions.

A non-conservative substitution leading to the formation of a functionally equivalent homologue of the sequences herein would for example i) differ substantially in polarity, for example a residue with a non-polar side chain (Ala, Leu, Pro, Trp, Val, Ile, Leu, Phe or Met) substituted for a residue with a polar side chain such as Gly, Ser, Thr, Cys, Tyr, Asn, or Gln or a charged amino acid such as Asp, Glu, Arg, or Lys, or substituting a charged or a polar residue for a non-polar one; and/or ii) differ substantially in its effect on polypeptide backbone orientation such as substitution of or for Pro or Gly by another residue; and/or iii) differ substantially in electric charge, for example substitution of a negatively charged residue such as Glu or Asp for a positively charged residue such as Lys, His or Arg (and vice versa); and/or iv) differ substantially in steric bulk, for example substitution of a bulky residue such as His, Trp, Phe or Tyr for one having a minor side chain, e.g. Ala, Gly or Ser (and vice versa).

Substitution of amino acids may in one embodiment be made based upon their hydrophobicity and hydrophilicity values and the relative similarity of the amino acid side-chain substituents, including charge, size, and the like. Exemplary amino acid substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In a preferred embodiment the binding domain comprises a homologue having an amino acid sequence at least 60 % homologous to SEQ ID NO 1.

More preferably the homology is at least 65 %, such as at least 70 % homologous, such as at least 75 % homologous, such as at least 80 % homologous, such as at least 85 % homologous, such as at least 90 % homologous, such as at least 95 % homologous, such as at least 98 % homologous to SEQ ID NO 1.

In a more preferred embodiment the percentages mentioned above relates to the identity of the sequence of a homologue as compared to SEQ ID NO 1.

Homologues to SEQ ID NO: 1 may be 27 aa human ghrelin SEQ ID NO: 2, rat ghrelin SEQ ID NO: 3. Additional preferred sequences are listed in EP 1 197 496 (Kangawa).

Other homologues are the variants described in EP 1197496 (Kangawa) incorporated herein by reference.

Bulky hydrophobic group

The bulky hydrophobic group of the secretagogue according to the invention is any bulky hydrophobic group capable of providing the des-acylated 28 aa human ghrelin, or an analogue thereof, with binding affinity to GHS-R 1a. Any suitable amino acid may be modified with any suitable bulky hydrophobic group; in a preferred embodiment, a Ser residue (preferably amino acid number 3 in the amino acid chain) is modified with the bulky hydrophobic group.

When the amino acid being modified contains e.g. -OH, -SH, -NH or $-NH_2$ as a substituent group in a side chain thereof, a group formed by acylating such a substituent group is preferred. The mode of linkage may thus be selected from the group consisting of ester, ether, thioester, thioether, amide and carbamide.

For example, if the modified amino acid is serine, threonine, tyrosine or oxyproline, the amino acid has a hydroxyl group in the side chain. If the modified amino acid is cysteine, the amino acid has a mercapto group in the side chain. If the modified amino acid is lysine, arginine, histidine, tryptophan, proline oroxyproline, it has an amino group or imino group in the side chain.

The hydroxyl group, mercapto group, amino group and imino group described above may thus have been chemically modified. That is, the hydroxyl group or mercapto group may be etherized, esterified, thioetherified or thioesterified. The imino group may have been iminoetherified, iminothioetherified or alkylated. The amino group may have been amidated, thioamidated or carbamidated.

Further, the mercapto group may have been disulfidated, the imino group may have been amidated or thioamidated, and the amino group may have been alkylated or thiocarbamidated.

In a preferred embodiment the modified amino acid is Ser coupled through an ester linkage to the hydrophobic group.

The hydrophobic group may be any group with a saturated or unsaturated alkyl or acyl group containing one or more carbon atoms. In one embodiment the bulky hydrophobic group is an acyl group, including groups formed by removing a hydroxyl group from an organic carboxylic acid, organic sulfonic acid or organic phosphoric acid. The organic carboxylic acid includes e.g. fatty acids, and the number of carbon atoms thereof is preferably 1 to 35. In the organic sulfonic acid or organic phosphoric acid, the number of carbon atoms thereof is preferably 1 to 35.

Accordingly, the acyl group is preferably selected from a C1-C35 acyl group, such as a C1 – C20 acyl group, such as a C1 – C15 acyl group, such as a C6 – C15 acyl group, such as a C6 – C12 acyl group, such as a C8 – C12 acyl group.

More preferably the acyl group is selected from the group of C7 acyl group, C8 acyl group, C9 acyl group, C10 acyl group, C11 acyl group, and C12 acyl group. Such acyl group may be formed from octanoic acid (preferably caprylic acid), decanoic acid (preferably capric acid), or dodecanoic acid (preferably lauric acid), as well as monoene or polyene fatty acids thereof.

In one embodiment the acyl group is selected from the group of C8 acyl group, and C10 acyl group. Such acyl groups may be formed from octanoic acid (preferably caprylic acid), or decanoic acid (preferably capric acid).

In another embodiment the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

Furthermore, the modified amino acid may be any amino acid wherein a group is modified as described in EP 1 197 496 (Kangawa), which is hereby incorporated by reference.

Protecting group

The ghrelin-like compound according to the invention may comprise a protecting group at the N-terminus or the C-terminus or at both.

A protecting group covalently joined to the N-terminal amino group reduces the reactivity of the amino terminus under in vivo conditions. Amino protecting groups include - C1-10 alkyl, -C1-10 substituted alkyl, -C2-10 alkenyl, -C2-10 substituted alkenyl, aryl, -C1-6 alkyl aryl, -C(O)- (CH2) 1-6-COOH, -C(O)-C1-6 alkyl, -C(O)-aryl, -C (O)-O-C1-6 alkyl, or-C (O)-O-aryl. Preferably, the amino terminus protecting group is acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or tbutyloxycarbonyl.

A protecting group covalently joined to the C-terminal carboxy group reduces the reactivity of the carboxy terminus under in vivo conditions. The carboxy terminus protecting group is preferably attached to the a-carbonyl group of the last amino acid. Carboxy terminus protecting groups include amide, methylamide, and ethylamide.

Conjugates

In another aspect of the present invention, a secretagogue, such as a ghrelin-like compound, may also be administered in a form, wherein the secretagogue compound is conjugated to another entity.

For example the compound may be a conjugate of ghrelin or a derivative or homologue thereof and another peptide, such as a peptide having effect on nociceptin receptor ORL1. In one embodiment the conjugate is a a conjugate of ghrelin or a derivative or homologue thereof and Ac-RYY(RK)(WI)RK)-NH_2, where the brackets show allowable variation of amino acid residues. Examples of peptides in the conjugate may also be found in US patent applications 2003040472 and US2002004483, and US patent 5869046.

In another preferred embodiment, said secretagogue conjugate includes reactive groups which can react with available reactive functionalities on blood components to form covalent bonds. The invention also relates to such modifications, such combinations with blood components and methods for their use. These methods include extending the effective therapeutic in vivo half life of the modified secretagogues. To

form covalent bonds with the functional group on a protein, one may use as a chemically reactive group (reactive entity) a wide variety of active carboxyl groups, particularly esters, where the hydroxyl moiety is physiologically acceptable at the levels required to modify the secretagogues. While a number of different hydroxyl groups may be employed in these linking agents, the most convenient would be N-hydroxysuccinimide (NHS), N-hydroxy-sulfosuccinimide (sulfo-NHS), maleimide-benzoyl-succinimide (MBS), gamma-maleimido-butyryloxy succinimide ester (GMBS) and maleimidopropionic acid (MPA). Primary amines are the principal targets for NHS esters. Accessible alpha-amine groups present on the N-termini of proteins react with NHS esters. However, alpha-amino groups on a protein may not be desirable or available for the NHS coupling. While five amino acids have nitrogen in their side chains, only the epsilon-amine of lysine reacts significantly with NHS esters. An amide bond is formed when the NHS ester conjugation reaction reacts with primary amines releasing N-hydroxysuccinimide as demonstrated in the schematic below. These succinimide containing reactive groups are herein referred to as succinimidyl groups. In one preferred embodiment of the present invention, the functional group on the protein will be a thiol group and the chemically reactive group will be a maleimido-containing group such as (GMBA or MPA). GMBA stands for gamma-maleimide-butrylamide. Such maleimide containing groups are referred to herein as maleimido groups. The maleimido group is most selective for sulfhydryl groups on peptides when the pH of the reaction mixture is kept between 6.5 and 7.4. At pH 7.0, the rate of reaction of maleimido groups with sulfhydryls is 1000-fold faster than with amines. A stable thioether linkage between the maleimido group and the sulfhydryl is formed which cannot be cleaved under physiological conditions.

In one preferred embodient, the secretagogues of this invention are designed to specifically react with thiol groups on mobile blood proteins. Such a reaction is preferably established by covalent bonding of a therapeutic peptide modified with a maleimide link (e.g. prepared from GMBS, MPA or other maleimides) to a thiol group on a mobile blood protein such as serum albumin or IgG.

Further, even among free thiol-containing blood proteins, using maleimides leads to the preferential formation of secretagogue-maleimide-albumin conjugates, due to the unique characteristics of albumin itself. The single free thiol group of albumin, highly conserved among species, is located at amino acid residue 34 (Cys_{34}). It has

been demonstrated recently that the Cys_{34} of albumin has increased reactivity relative to free thiols on other free thiol-containing proteins. This is due in part to the very low pK value of 5.5 for the Cys_{34} of albumin. This is much lower than typical pK values for cysteines residues in general, which are typically about 8. Due to this low pK, under normal physiological conditions Cys_{34} of albumin is predominantly in the ionized form, which dramatically increases its reactivity, as reported in. In addition to the low pK value of Cys_{34}, another factor which enhances the reactivity of Cys_{34} is its location, which is in a crevice close to the surface of one loop of region V of albumin. This location makes Cys_{34} very available to ligands of all kinds, and is an important factor in Cys_{34}'s biological role as free radical trap and free thiol scavenger. These properties make Cys_{34} highly reactive with secretagogue-maleimides, and the reaction rate acceleration can be as much as 1000-fold relative to rates of reaction of secretagogue-maleimides with other free-thiol containing proteins.

In contrast to NHS-peptides, maleimide-secretagogue conjugates are generally quite stable in the presence of aqueous solutions and in the presence of free amines. Since maleimide-conjugates will only react with free thiols, protective groups are generally not necessary to prevent the maleimide-secretagogue conjugate from reacting with itself. In addition, the increased stability of the peptide permits the use of further purification steps such as HPLC to prepare highly purified products suitable for in vivo use. Lastly, the increased chemical stability provides a product with a longer shelf life.

The desired conjugates of non-specific secretagogues to blood components may be prepared in vivo by administration of the secretagogues directly to the patient, which may be a human or other mammal. The administration may be done in any suitable form, such as in the form of a bolus or introduced slowly over time by infusion using metered flow or the like.

If desired, the subject conjugates may also be prepared ex vivo by combining blood with derivatized secretagogues of the present invention, allowing covalent bonding of the modified secretagogues to reactive functionalities on blood components and then returning or administering the conjugated blood to the host. Moreover, the above may also be accomplished by first purifying an individual blood component or limited number of components, such as red blood cells, immunoglobulins, serum

albumin, or the like, and combining the component or components ex vivo with the chemically reactive secretagogues.

In the following sections "Conjugation to a lipophilic compound", "Conjugation to a polymer molecule", "Conjugation to an oligosaccharide moiety" and "Conjugation to an organic derivatizing agent" conjugation to specific types of non-polypeptide moieties is described. In general, a secretagogue conjugate according to the invention may be produced by culturing an appropriate host cell under conditions conducive for expression of the secretagogue polypeptide, and recovering the polypeptide, wherein a) the polypeptide comprises at least one N- or O-glycosylation site and the host cell is a eukaryotic host cell capable of in vivo glycosylation, and/or b) the polypeptide is subjected to conjugation to a non-polypeptide moiety in vitro. Said glycosylation may be via synthetic means as well as natural means.

Conjugation of a secretagogue compound to a Lipophilic Compound

The secretagogue polypeptide and the lipophilic compound may be conjugated to each other, either directly or by use of a linker. The lipophilic compound may be a natural compound such as a saturated or unsaturated fatty acid, a fatty acid diketone, a terpene, a prostaglandin, a vitamin, a carotenoid or steroid, or a synthetic compound such as a carbon acid, an alcohol, an amine and sulphonic acid with one or more alkyl, aryl, alkenyl or other multiple unsaturated compounds. The conjugation between the polypeptide and the lipophilic compound, optionally through a linker, may be done according to methods known in the art, e.g., as described by Bodanszky in Peptide Synthesis, John Wiley, New York, 1976 and in WO 96/12505.

Conjugation of a secretagogue compound to a polymer molecule

The polymer molecule to be coupled to the polypeptide may be any suitable polymer molecule, such as a natural or synthetic homo-polymer or heteropolymer, typically with a molecular weight in the range of about 300-100,000 Da, such as about 500-20,000 Da, more preferably in the range of about 1000-15,000 Da, even more preferably in the range of about 2000-12,000 Da, such as about 3000-10,000. When used about polymer molecules herein, the word "about" indicates an approximate average molecular weight and reflects the fact that there will normally be a certain molecular weight distribution in a given polymer preparation.

Examples of homo-polymers include a polyol (i.e., poly-OH), a polyamine (i.e., poly-NH2) and a polycarboxylic acid (i.e., poly-COOH). A hetero-polymer is a polymer which comprises different coupling groups, such as a hydroxyl group and an amine group.

Examples of suitable polymer molecules include polymer molecules selected from the group consisting of polyalkylene oxide (PAO), including polyalkylene glycol (PAG), such as linear or branched polyethylene glycol (PEG) and polypropylene glycol (PPG), poly-vinyl alcohol (PVA), poly-carboxylate, poly-(vinylpyrolidone), polyethylene-co-maleic acid anhydride, polystyrene-co-maleic acid anhydride, dextran, including carboxymethyl-dextran, or any other biopolymer suitable for reducing immunogenicity and/or increasing functional in vivo half-life and/or serum half-life. Another example of a polymer molecule is human albumin or another abundant plasma protein. Generally, polyalkylene glycol-derived polymers are biocompatible, non-toxic, non-antigenic, non-immunogenic, have various water solubility properties, and are easily excreted from living organisms.

PEG is one preferred polymer molecule, since it has only few reactive groups capable of cross-linking compared to polysaccharides such as dextran. In particular, monofunctional PEG, e.g., methoxypolyethylene glycol (mPEG), is of interest since its coupling chemistry is relatively simple (only one reactive group is available for conjugating with attachment groups on the polypeptide). Consequently, the risk of cross-linking is eliminated, the resulting polypeptide conjugates are more homogeneous and the reaction of the polymer molecules with the polypeptide is easier to control.

To effect covalent attachment of the polymer molecule(s) to the polypeptide, the hydroxyl end groups of the polymer molecule are provided in activated form, i.e., with reactive functional groups. Suitable activated polymer molecules are commercially available, e.g., from Shearwater Polymers, Inc., Huntsville, AL, USA, or from PolyMASC Pharmaceuticals plc, UK. Alternatively, the polymer molecules can be activated by conventional methods known in the art, e.g., as disclosed in WO 90/13540. Specific examples of activated linear or branched polymer molecules for use in the present invention are described in the Shearwater Polymers, Inc. 1997 and 2000 Catalogs (Functionalized Biocompatible Polymers for Research and

pharmaceuticals, Polyethylene Glycol and Derivatives, incorporated herein by reference). Specific examples of activated PEG polymers include the following linear PEGs: NHS-PEG (e.g., SPA-PEG, SSPA-PEG, SBA-PEG, SS-PEG, SSA-PEG, SC-PEG, SG-PEG, and SCM-PEG), and NOR-PEG), BTC-PEG, EPOX-PEG, NCO-PEG, NPC-PEG, CDI-PEG, ALD-PEG, TRES-PEG, VS-PEG, IODO-PEG, and MAL-PEG, and branched PEGs such as PEG2-NHS and those disclosed in U.S. Pat. No. 5,932,462 and U.S. 5,643,575, both of which are incorporated herein by reference.

Furthermore, the following publications, incorporated herein by reference, disclose useful polymer molecules and/or PEGylation chemistries: U.S. Pat. No. 5,824,778, U.S. 5,476,653, WO 97/32607, EP 229,108, EP 402,378, U.S. Pat. No. 4,902,502, U.S. 5,281,698, U.S. 5,122,614, U.S. 5,219,564, WO 92/16555, WO 94/04193, WO 94/14758, WO 94/17039, WO 94/18247, WO 94/28024, WO 95/00162, WO 95/11924, WO95/13090, WO 95/33490, WO 96/00080, WO 97/18832, WO 98/41562, WO 98/48837, WO 99/32134, WO 99/32139, WO 99/32140, WO 96/40791, WO 98/32466, WO 95/06058, EP 439 508, WO 97/03106, WO 96/21469, WO 95/13312, EP 921 131, U.S. Pat. No. 5,736,625, WO 98/05363, EP 809 996, U.S. Pat. No. 5,629,384, WO 96/41813, WO 96/07670, U.S. Pat. No. 5,473,034, U.S. 5,516,673, EP 605 963, U.S. Pat. No. 5,382,657, EP 510 356, EP 400 472, EP 183 503 and EP 154 316.

The conjugation of the secretagogue polypeptide and the activated polymer molecules is conducted by use of any conventional method, e.g., as described in the following references (which also describe suitable methods for activation of polymer molecules): R. F. Taylor, (1991), "Protein immobilisation. Fundamental and applications", Marcel Dekker, N.Y.; S. S. Wong, (1992), "Chemistry of Protein Conjugation and Crosslinking", CRC Press, Boca Raton; G. T. Hermanson et al., (1993), "Immobilized Affinity Ligand Techniques", Academic Press, N.Y.). The skilled person will be aware that the activation method and/or conjugation chemistry to be used depends on the attachment group(s) of the polypeptide (examples of which are given further above), as well as the functional groups of the polymer (e.g., being amine, hydroxyl, carboxyl, aldehyde, sulfydryl, succinimidyl, maleimide, vinysulfone or haloacetate). The PEGylation may be directed towards conjugation to all available attachment groups on the polypeptide (i.e., a bulky hydrophobic group or such attachment groups that are exposed at the surface of the polypeptide) or may be di-

rected towards one or more specific attachment groups, e.g., the N-terminal amino group (U.S. Pat. No. 5,985,265). Furthermore, the conjugation may be achieved in one step or in a stepwise manner (e.g., as described in WO 99/55377).

It will be understood that the PEGylation is designed so as to produce the optimal molecule with respect to the number of PEG molecules attached, the size and form of such molecules (e.g., whether they are linear or branched), and where in the polypeptide such molecules are attached. The molecular weight of the polymer to be used will be chosen taking into consideration the desired effect to be achieved. For instance, if the primary purpose of the conjugation is to achieve a conjugate having a high molecular weight and larger size (e.g., to reduce renal clearance), one may choose to conjugate either one or a few high molecular weight polymer molecules or a number of polymer molecules with a smaller molecular weight to obtain the desired effect. Preferably, however, several polymer molecules with a smaller molecular weight will be used. When a high degree of epitope shielding is desirable, this may be obtained by use of a sufficiently high number of low molecular weight polymer molecules (e.g., with a molecular weight of about 5,000 Da) to effectively shield all or most epitopes of the polypeptide. For instance, 2-8, such as 3-6 such polymers may be used. As the examples below illustrate, it may be advantageous to have a larger number of polymer molecules with a lower molecular weight (e.g., 4-6 with a MW of 5000) compared to a smaller number of polymer molecules with a higher molecular weight (e.g., 1-3 with a MW of 12,000-20,000) in terms of improving the functional in vivo half-life of the polypeptide conjugate, even where the total molecular weight of the attached polymer molecules in the two cases is the same. It is believed that the presence of a larger number of smaller polymer molecules provides the polypeptide with a larger diameter or apparent size than e.g., a single yet larger polymer molecule, at least when the polymer molecules are relatively uniformly distributed on the polypeptide surface.

While conjugation of only a single polymer molecule to a single attachment group on the protein is not the most preferred embodiment, in the event that only one polymer molecule is attached, it will generally be advantageous that the polymer molecule, which may be linear or branched, has a relatively high molecular weight, e.g., about 20 kDa.

Normally, the polymer conjugation is performed under conditions aiming at reacting as many of the available polymer attachment groups as possible with polymer molecules. This is achieved by means of a suitable molar excess of the polymer in relation to the polypeptide. Typical molar ratios of activated polymer molecules to polypeptide are up to about 1000-1, such as up to about 200-1 or up to about 100-1. In some cases, the ratio may be somewhat lower, however, such as up to about 50-1, 10-1 or 5-1.

It is also contemplated according to the invention to couple the polymer molecules to the polypeptide through a linker. Suitable linkers are well known to the skilled person. A preferred example is cyanuric chloride (Abuchowski et al., (1977), J. Biol. Chem., 252, 3578-3581; U.S. Pat. No. 4,179,337; Shafer et al., (1986), J. Polym. Sci. Polym. Chem. Ed., 24, 375-378.

Subsequent to the conjugation residual activated polymer molecules are blocked according to methods known in the art, e.g., by addition of primary amine to the reaction mixture, and the resulting inactivated polymer molecules are removed by a suitable method (see Materials and Methods).

In a preferred embodiment, the polypeptide conjugate of the invention comprises a PEG molecule attached to some, most or preferably substantially all of the lysine residues in the polypeptide available for PEGylation, in particular a linear or branched PEG molecule, e.g., with a molecular weight of about 1-15 kDa, typically about 2-12 kDa, such as about 3-10 kDa, e.g., about 5 or 6 kDa.

It will be understood that depending on the circumstances, e.g., the amino acid sequence of the polypeptide, the nature of the activated PEG compound being used and the specific PEGylation conditions, including the molar ratio of PEG to polypeptide, varying degrees of PEGylation may be obtained, with a higher degree of PEGylation generally being obtained with a higher ratio of PEG to polypeptide. The PEGylated polypeptides resulting from any given PEGylation process will, however, normally comprise a stochastic distribution of polypeptide conjugates having slightly different degrees of PEGylation.

In yet another embodiment, the secretagogue conjugate of the invention may comprise a PEG molecule attached to the lysine residues in the polypeptide available for PEGylation, and in addition to the N-terminal amino acid residue of the polypeptide.

Coupling to an Oligosaccharide Moiety

Covalent in vitro coupling of glycosides (such as dextran) to amino acid residues of the polypeptide may also be used, e.g., as described in WO 87/05330 and in Aplin et al., CRC Crit Rev. Biochem., pp. 259-306, 1981.

The *in vitro* coupling of oligosaccharide moieties or PEG to protein- and peptide-bound Gln-residues such as the secretagogues used in the present invention can be carried out by transglutaminases (TG'ases). Transglutaminases catalyse the transfer of donor amine-groups to protein- and peptide-bound Gln-residues in a so-called cross-linking reaction. The donor-amine groups can be protein- or peptide-bound e.g., as the epsilon-amino-group in Lys-residues or can be part of a small or large organic molecule. An example of a small organic molecule functioning as an amino-donor in TG'ase-catalysed cross-linking is putrescine (1,4-diaminobutane). An example of a larger organic molecule functioning as an amino-donor in TG'ase-catalysed cross-linking is an amine-containing PEG (Sato et al., Biochemistry 35, 13072-13080).

Tg'ases are in general highly specific enzymes, and not every Gln-residue exposed on the surface of a protein is accessible to TG' ase-catalysed cross-linking to amino-containing substances. On the contrary, only a few Gln-residues function naturally as TG'ase substrates, but the exact parameters governing which Gln-residues are good TG'ase substrates remain unknown. Thus, in order to render a protein, such as a secretagogue, susceptible to TG'ase-catalysed cross-linking reactions, it is often a prerequisite to add at convenient positions stretches of amino acid sequence known to function very well as TG'ase substrates. Several amino acid sequences are known to be or to contain excellent natural TG'ase substrates e.g., substance P, elafin, fibrinogen, fibronectin, alpha2-plasmin inhibitor, alpha-caseins, and beta-caseins.

Coupling to an Organic Derivatizing Agent

Covalent modification of the secretagogue may be performed by reacting one or more attachment groups of the secretagogue with an organic derivatizing agent. Suitable derivatizing agents and methods are well known in the art. For example, cysteinyl residues most commonly are reacted with alpha-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, alpha-bromo-beta-(4-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole. Histidyl residues are derivatized by reaction with diethylpyrocarbonateat pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide is also useful. The reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0. Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione and transaminase-catalyzed reaction with glyoxylate. Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine guanidino group. Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R-N=C=N-R'), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Conjugation to the Fc region of an IgG

In one preferred embodiment of the present invention, a salvage receptor binding epitope of the Fc region of an IgG (i.e. the Fc portion of an immunoglobulin of the isotype IgG) is incorporated into a secretagogue so as to increase its circulatory

half-life, but so as not to lose its biological activity. This can take place by any means, such as by mutation of the appropriate region in the secretagogue to mimic the Fc region or by incorporating the epitope into a peptide tag that is then fused to the secretagogue at either end or in the middle or by DNA or peptide synthesis.

A systematic method for preparing such a secretagogue conjugate having an increased in vivo half-life comprises several steps. The first involves identifying the sequence and conformation of a salvage receptor binding epitope on an Fc region of an IgG molecule. Once this epitope is identified, the sequence of the secretagogue is modified to include the sequence and conformation of the identified binding epitope. After the sequence is mutated, the secretagogue varient is tested to see if it has a longer in vivo half-life than that of the original secretagogue. If the variant does not have a longer in vivo half-life upon testing, its sequence is further altered to include the sequence and conformation of the identified binding epitope. The altered polypeptide is tested for longer in vivo half-life, and this process is continued until a molecule is obtained that exhibits a longer in vivo half-life.

The salvage receptor binding epitope being thus incorporated into the secretagogue is any suitable such epitope as defined above, and its nature will depend, e.g., on the type of secretagogue being modified. The transfer is made such that the biological activity of the secretagogue is maintained, i.e., the transferred portion does not adversely affect the conformation of the secretagogue or affect its binding to ligands that confers its biological activity.

Method for production

Ghrelin-like compounds can be produced using techniques well known in the art. For example, a polypeptide region of a ghrelin-like compound can be chemically or biochemically synthesized and modified. Techniques for chemical synthesis of polypeptides are well known in the art. (See e. g., Vincent in Peptide and Protein Drug Delivery, New York, N. Y., Dekker, 1990.) Examples of techniques for biochemical synthesis involving the introduction of a nucleic acid into a cell and expression of nucleic acids are provided in Ausubel, Current Protocols in Molecular Biology, John Wiley, 1987-1998, and Sambrook et al., in Molecular Cloning, A

Laboratory Manual, 2 d Edition, Cold Spring Harbor Laboratory Press, 1989.

Pharmaceutical composition

In one aspect the invention relates to a pharmaceutical composition comprising a ghrelin-like compound as defined herein, wherein the bulky hydrophobic group is an acyl group selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

In another embodiment the invention relates to a pharmaceutical composition comprising a mixture of at least two different ghrelin-like compounds, such as a mixture of a ghrelin-like compound being acylated with a C8 acyl and a ghrelin-like compound being acylated with a C10 acyl. Without being bound by theory it is believed that such a mixture will have a longer half-life in plasma.

In yet another embodiment, the pharmaceutical composition comprises acylated ghrelin-like compounds, optionally compounds having different acyl chain lengths preferably selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group, further optionally in combination with a desacylated Ghrelin-like compound.

In another aspect the invention relates to a pharmaceutical composition comprising any compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients said composition further comprising transport molecules. The transport molecules are primarily added in order to increase the half-life of the acylated compound, preventing premature des-acylation, since the des-acylated ghrelin is not active at the GHS-R 1a.

Transport molecules act by having incorporated into or anchored to it the compound according to the invention.

Any suitable transport molecules known to the skilled person may be used. Examples of transport molecules may be liposomes, micelles, and/or microspheres.

A variety of methods are available for preparing liposomes, as described in, e.g., Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467 (1980), U.S. Pat. Nos. 4, 235,871, 4,501,728 and 4,837,028, all of which are incorporated herein by reference. One method produces multilamellar vesicles of heterogeneous sizes. In this method, the vesicle-forming lipids are dissolved in a suitable organic solvent or solvent system and dried under vacuum or an inert gas to form a thin lipid film. If desired, the film may be redissolved in a suitable solvent, such as tertiary butanol, and then lyophilized to form a more homogeneous lipid mixture which is in a more easily hydrated powderlike form. This film is covered with an aqueous solution of the targeted drug and the targeting component and allowed to hydrate, typically over a 15-60 minute period with agitation. The size distribution of the resulting multilamellar vesicles can be shifted toward smaller sizes by hydrating the lipids under more vigorous agitation conditions or by adding solubilizing detergents such as deoxycholate. Additionally, the liposome suspension may include lipid-protective agents which protect lipids against free-radical and lipid-peroxidative damages on storage. Lipophilic free-radical quenchers, such as alpha-tocopherol and water-soluble iron-specific chelators, such as ferrioxianine, are preferred.

Micelles are formed by surfactants (molecules that contain a hydrophobic portion and one or more ionic or otherwise strongly hydrophilic groups) in aqueous solution. As the concentration of a solid surfactant increases, its monolayers adsorbed at the air/water or glass/water interfaces become so tightly packed that further occupancy requires excessive compression of the surfactant molecules already in the two monolayers. Further increments in the amount of dissolved surfactant beyond that concentration cause amounts equivalent to the new molecules to aggregate into micelles. This process begins at a characteristic concentration called "critical micelle concentration".

The shape of micelles formed in dilute surfactant solutions is approximately spherical. The polar head groups of the surfactant molecules are arranged in an outer spherical shell whereas their hydrocarbon chains are oriented toward the center, forming a spherical core for the micelle. The hydrocarbon chains are randomly coiled and entangled and the micellar interior has a nonpolar, liquid-like character. In the micelles of polyoxyethylated nonionic detergents, the polyoxyethlene moieties are oriented outward and permeated by water. This

arrangement is energetically favorable since the hydrophilic head groups are in contact with water and the hydrocarbon moieties are removed from the aqueous medium and partly shielded from contact with water by the polar head groups. The hydrocarbon tails of the surfactant molecules, located in the interior of the micelle, interact with one another by weak van der Waals forces.

The size of a micelle or its aggregation number is governed largely by geometric factors. The radius of the hydrocarbon core cannot exceed the length of the extended hydrocarbon chain of the surfactant molecule. Therefore, increasing the chain length or ascending homologous series increases the aggregation number of spherical micelles. If the surfactant concentration is increased beyond a few percent and if electrolytes are added (in the case of ionic surfactants) or the temperature is raised (in the case of nonionic surfactants), the micelles increase in size. Under these conditions, the micelles are too large to remain spherical and become ellipsoidal, cylindrical or finally lamellar in shape.

Common surfactants well known to one of skill in the art can be used in the micelles of the present invention. Suitable surfactants include sodium laureate, sodium oleate, sodium lauryl sulfate, octaoxyethylene glycol monododecyl ether, octoxynol 9 and PLURONIC F-127 (Wyandotte Chemicals Corp.). Preferred surfactants are nonionic polyoxyethylene and polyoxypropylene detergents compatible with IV injection such as, TWEEN-80., PLURONIC F-68., n-octyl-.beta.-D-glucopyranoside, and the like. In addition, phospholipids, such as those described for use in the production of liposomes, may also be used for micelle formation.

In another preferred embodiment, the compounds of the present invention are formulated as described in the literature for an administration route selected from: buccal delivery, sublingual delivery, transdermal delivery, inhalation and needle-free injection, such as using the methods developed by Powderjet.

For inhalation, the compounds of the present invention can be formulated as using methods known to those skilled in the art, for example an aerosol, dry powder or solubolized such as in microdroblets, preferably in a device intended for such delivery (such as commercially available from Aradigm, Alkerme or Nektar).

Administration

Suitable dosing regimens are preferably determined taking into account factors well known in the art including type of subject being dosed; age, weight, sex and medical condition of the subject; the route of administration; the renal and hepatic function of the subject; the desired effect; and the particular compound employed.

Optimal precision in achieving concentrations of drug within the range that yields efficacy without toxicity requires a regimen based on the kinetics of the drug's availability to target sites. This involves a consideration of the distribution, equilibrium, and elimination of a drug.

As described above, in one aspect of the invention, the ghrelin-like compound is administered subcutaneously.

In another aspect the ghrelin-like compound is administered as a premeal bolus, wherein the administration form may be any suitable parenteral form.

In a preferred embodiment the ghrelin-like compound is administered subcutaneously in a premeal bolus.

The ghrelin-like compound can also be administered during a meal as a bolus. The mode of administration during a meal includes subcoutaneous administration, such as a subcoutaneously administered bolus.

Pharmaceutical compositions for parenteral administration include sterile aqueous and non-aqueous injectable solutions, dispersions, suspensions or emulsions, as well as sterile powders to be reconstituted in sterile injectable solutions or dispersions prior to use.

Other suitable administration forms include suppositories, sprays, ointments, cremes, gels, inhalants, dermal patches, implants, pills, tablets, lozenges and capsuls.

A typical dosage of a compound employed according to the invention is in a con-

centration equivalent to from 10 ng to 10 mg ghrelin per kg bodyweight. The concentrations and amounts herein are given in equivalents of amount ghrelin, wherein the ghrelin is the 28 aa human ghrelin. Equivalents may be tested as described in Example 2.

In a preferred embodiment the medicament is administered in a concentration equivalent to from 0.1 µg to 1 mg ghrelin per kg bodyweight, such as from 0.5 µg to 0.5 mg ghrelin per kg bodyweight, such as from 1.0 µg to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 µg to 50 µg ghrelin per kg bodyweight, such as from 1.0 µg to 10 µg ghrelin per kg bodyweight.

As described above, the ghrelin-like compound is preferably administered as a bolus. Accordingly, in one embodiment the medicament is administered as a bolus prior to a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin. More preferred the medicament is administered as a bolus prior to a meal, said bolus comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 2.0 µg to 200 mg ghrelin, such as from 5.0 µg to 100 mg ghrelin, such as from 10 µg to 50 mg ghrelin, such as from 10 µg to 5 mg ghrelin, such as from 10 µg to 1.0 mg ghrelin.

It should be noted that the normal ghrelin response which occurs before a meal is a short-lived surge in plasma concentrations of ghrelin and that due to the relative short half life of the peptide an i.v. injection of ghrelin will ensure that a similar short-lived peak on ghrelin concentrations can be obtained. The administration route must ensure that the non-degraded, bioactive form of the peptide will be the dominating form in the circulation, which will reach the ghrelin receptors and stimulate these. Thus, in order to obtain the maximum effect of the medicament it is preferably administered from one to three times daily, each administration being within 45 minutes of a meal, such as within 30 minutes of a meal, such as within 25 minutes of a meal, such as within 20 minutes of a meal, such as within 15 minutes of a meal, such as within 10 minutes of a meal, such as within 5 minutes of a meal. More preferred the medicament is administered prior to each main meal, such as administered three times daily.

Formulation

In a preferred aspect the present invention contemplates pharmaceutical compositions useful for practicing the therapeutic methods described herein. Pharmaceutical compositions of the present invention contain a physiologically tolerable carrier together with at least one species of ghrelin-like compound as described herein, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the pharmaceutical composition is not immunogenic when administered to a human individual for therapeutic purposes, unless that purpose is to induce an immune response.

In one aspect the invention relates to a pharmaceutical composition comprising at least one ghrelin-like compound as defined above. In a preferred embodiment the pharmaceutical composition comprises at least two different ghrelin-like compounds as defined above in order to increase the effect of the treatment. The difference may for example be compounds having different acylations as discussed above.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a human without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art. Typically such compositions are prepared as sterile injectables either as liquid solutions or suspensions, aqueous or non-aqueous, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

The pharmaceutical composition of the present invention can include pharmaceutically acceptable salts of the compounds therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the polypeptide).

Such salts include pharmaceutically acceptable acid addition salts, pharmaceutically acceptable metal salts, ammonium salts and alkylated ammonium salts. Acid addition salts include salts of inorganic acids as well as organic acids. Representative examples of suitable inorganic acids include hydrochloric, hydrobromic, hydriodic, phosphoric, sulpfuric and nitric acids and the like. Representative examples of suitable organic acids include formic, acetic, trichloroacetic, trifluoroacetic, propionic, benzoic, cinnamic, citric, fumaric, glycolic, lactic, maleic, malic, malonic, mandelic, oxalic, picric, pyruvic, salicylic, succinic, methanesulfonic, ethanesulfonic, tartaric, ascorbic, pamoic, bismethylene salicylic, ethanedisulfonic, gluconic, citraconic, aspartic, stearic, palmitic, ethylenediaminetetraacetic (EDTA), p-aminobenzoic, glutamic, benzenesulfonic and ptoluenesulfonic acids and the like. Further examples of pharmaceutically acceptable inorganic or organic acid addition salts include the pharmaceutical acceptable salts listed in J. Pharm. Sci. 1977,66,2, which is incorporated herein by reference. Examples of metal salts include lithium, sodium, potassium and magnesium salts and the like.

Examples of ammonium and alkylated ammonium salts include ammonium, methylammonium, dimethylammonium, trimethylammonium, ethylammonium, hydroxyethylammonium, diethylammonium, butylammonium and tetramethylammonium salts and the like.

Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

Also included within the scope of compounds or pharmaceutical acceptable acid addition salts thereof in the context of the present invention are any hydrates (hydrated forms) thereof.

For parenteral administration, solutions of the present compounds in sterile aqueous solution, aqueous propylene glycol or sesame or peanut oil may be employed. Such aqueous solutions should be suitably buffered if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. The aqueous solutions are particularly suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. The sterile aqueous media employed are all readily available by standard techniques known to those skilled in the art.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, organic esters such as ethyl oleate, and water-oil emulsions.

Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. Examples of solid carriers are lactose, terra alba, sucrose, cyclodextrin, talc, gelatine, agar, pectin, acacia, magnesium stearate, stearic acid or lower alkyl ethers of cellulose. Examples of liquid carriers are syrup, peanut oil, olive oil, phospholipids, fatty acids, fatty acid amines, polyoxyethylene or water. Administered by nasal aerosol or inhalation formulations may be prepared, for example, as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, employing fluorocarbons, and/or employing other solubilizing or dispersing agents.

The pharmaceutical compositions formed by combining the compounds of the invention and the pharmaceutical acceptable carriers are then readily administered in a variety of dosage forms suitable for the disclosed routes of administration. The formulations may conveniently be presented in unit dosage form by methods known in the art of pharmacy.

In a preferred embodiment of the invention the formulation comprises the ghrelin-like compound or a salt thereof as a lyophilisate and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartements until administration.

In another embodiment the formulation is a solution of the ghrelin-like compound or a salt thereof.

In both embodiment the solvent may be any suitable solvents, such as described herein, and preferably the solvent is saline.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising an compound of the invention, comprising admixing at least one ghrelin-like compound as defined above with a physiologically acceptable carrier.

In a still further aspect, the invention relates to a pharmaceutical composition comprising, as an active ingredient, a compound as defined above or a pharmaceutical acceptable salt thereof together with a pharmaceutical acceptable carrier.

Accordingly, the formulation may further include the transport molecules as described above.

In a further aspect of the invention the present compounds may be administered in combination with further pharmacologically active substances, e. g. an antidiabetic agent or other pharmacologically active material, including other compounds for the treatment and/or prevention of insulin resistance and diseases wherein insulin resistance is the pathophysiological mechanism. The combination may be in the form of kit-in-part systems, wherein the combined active substances may be used for simultaneous, sequential or separate administration.

The ghrelin-like compounds according to the invention can be administered in combination with other appetite-regulating agents. The ghrelin-like compounds according to the invention can also be administered in combination with a pharmaceutically effective amount of a growth hormone, including hGH.

Uses and combination treatments involving administration of the ghrelin-like compound according to the invention can also involve treatment of one or more of

a) prophylaxis or treatment of cachexia, and/or

b) prophylaxis or treatment of lipodystrophy, and/or
c) stimulation of appetite, and/or
d) stimulation of food intake, and/or
e) stimulation of weight gain, and/or
f) increase of body fat mass,

including any individual condition, or combination of conditions as listed herein above, in combination with one or more of

g) prophylaxis and/or alleviation and/or treatment of a clinical depression, which combination treatment further comprises administering an antidepressant, a prodrug thereof, or a pharmaceutically acceptable salt of said antidepressant or said prodrug, and/or

h) prophylaxis and/or alleviation and/or treatment of an emetic condition, including nausia and vomiting, which combination treatment further comprises administering an antiemetic agent, a prodrug thereof, or a pharmaceutically acceptable salt of said antiemetic agent or said prodrug, and/or

i) prophylaxis and/or alleviation and/or treatment of a psychotic condition, which combination treatment further comprises administering an antipsychotic agent, a prodrug thereof or a pharmaceutically acceptable salt of said antipsychotic agent or said prodrug, and/or

j) prophylaxis and/or alleviation and/or treatment of anxiety, which combination treatment further comprises administering an antianxiety agent, a prodrug thereof or a pharmaceutically acceptable salt of said antianxiety agent or said prodrug, and/or

Clinical Depression

Clinical depression is characterized by a combination of symptoms that interfere with the ability to work, study, sleep, eat, and enjoy once pleasurable activities. Symptoms include: persistent sad or anxious mood; feelings of hopelessness or pessimism; feelings of guilt, worthlessness or helplessness; loss of interest in pleasure activities; decreased energy; difficulty concentrating, remembering, or making

decisions; sleep abnormalities (e.g. insomnia); appetite and/or weight loss; thoughts of death or suicide; restlessness; and irritability.

Depression is a common disorder, occurring in approximately 10 percent of the U.S. population. Major depression is a leading cause of disability in the U.S. and worldwide, and a leading cause of days lost from work. Many causes of clinical depression having roots in the anatomy of the human brain. Neurotransmitter activity, genetic predisposition, and environmental factors are believed to be involved in the development of depression.

Diagnosis of depression is complicated, requiring a physical examination to rule out certain medications or medical conditions and a psychological examination to thoroughly evaluate the symptoms and determine how severely the symptoms have affected the life of the patient. Accordingly, depression is difficult to diagnose due to the variety of ways in which depression manifests itself.

In the above combination treatment, the antidepressant is preferably a norepinephrine reuptake inhibitor (NERI), a selective serotonin reuptake inhibitor (SSRI), a monoamine oxidase inhibitor (MAO), a combined NERI/SSRI, or an atypical antidepressant, a prodrug of said antidepressant or a pharmaceutically acceptable salt of said antidepressant or said prodrug.

One preferred antidepressant is a selective serotonin reuptake inhibitor (SSRI), a prodrug thereof or a pharmaceutically acceptable salt of said SSRI or said prodrug. The SSRI is preferably citalopram, escitalopram, femoxetine, fluoxetine, fluvoxamine, indalpine, indeloxazine, milnacipran, paroxetine, sertraline, sibutramine or zimeldine, a prodrug of said SSRI or a pharmaceutically acceptable salt of said SSRI or said prodrug. Of the above, citalopram and escitalopram, a prodrug or a pharmaceutically acceptable salt thereof, are preferred in certain embodiments of combination treatments according to the present invention.

Emesis, including nausia and vomiting

Emesis can be caused by a number of factors, including medication. Emesis can thus be an unavoidable side effect associated with many forms of medication. Pre-

ferred antiemetic agents according to the invention include meclizine hydrochloride, prochlorperazine, promethazine, trimethobenzamide hydrochloride and ondansetron hydrochloride.

In particular emesis may be caused by cancer, either due to the anti-cancer treatment or due to the cancer disease as such.

Clinical Psychotic Condition

There are few psychiatric disorders in which clinical manifestations and symptoms can be correlated with a demonstrable pathology. The majority of mental illnesses are evaluated by observing changes in key behaviors such as thinking, mood, or social behavior. These alterations are difficult to ascertain and nearly impossible to quantify.

Preferred antipsychotic agents in accordance with the present invention are chlorpromazine, haloperidol, clozapine, loxapine, molindone hydrochloride, thiothixene, olanzapine, ziprasidone, ziprasidone hydrochloride, prochlorperazine, perphenazine, trifluoperazine hydrochloride and risperidone.

Anxiety

The combination methods of this invention are also directed to treatment, prevention, amelioration, alleviation, and of conditions of anxiety in a mammal, including a human being. The term anxiety covers generalized anxiety disorder, panic anxiety, obsessive compulsive disorder, social phobia, performance anxiety, post-traumatic stress disorder, acute stress reaction, adjustment disorders, hypochondriacal disorders, separation anxiety disorder, agoraphobia and specific phobias.

Preferred antianxiety agents are alprazolam, clonazepam, lorazepam, oxazepam, chlordiazepoxide hydrochloride, diazepam, buspirone hydrochloride, doxepin hydrochloride, hydroxyzine pamoate and clonazepam.

Further conditions of in individual capable of being treatable in accordance with the present invention are bulimia nervosa, male erectile dysfunction, female sexual

dysfunction, thyroid cancer, breast cancer, amelioration of ischemic nerve or muscle damage, as well as systemic lupus erythematosus.

The above medicaments are administered in pharmaceutically effective amounts, i.e. an administration involving a total amount of each active component of the medicament or pharmaceutical composition or method that is sufficient to show a meaningful patient benefit.

Medical packaging

The compounds of the invention may be administered alone or in combination with pharmaceutically acceptable carriers or excipients, in either single or multiple doses. The formulations may conveniently be presented in unit dosage form by methods known to those skilled in the art.

It is preferred that the compounds according to the invention is provided in a kit. Such a kit typically contains an active compound in dosage forms for administration. A dosage form contains a sufficient amount of active compound such that a desirable effect can be obtained when administered to a subject, preferably prior to at least one meal a day, more preferably prior to each main meal, such as three times a day, during the course of 1 or more days.

Thus, it is preferred that the medical packaging comprises an amount of dosage units corresponding to the relevant dosage regimen. Accordingly, in one embodiment, the medical packaging comprises a pharmaceutical composition comprising a compound as defined above or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients, said packaging having from 7 to 21 dosage units, or multipla thereof, thereby having dosage units for one week of administration or several weeks of administration.

In one embodiment the medical packaging is for administration once daily in a week, and comprises 7 dosage units, in another embodiment the medical packaging is for administration twice daily, and comprises 14 dosage units. In yet another more preferred embodiment the medical packaging is for administration three times daily, and comprises 21 dosage units.

The dosage units are as defined above, ie. a dosage unit preferably comprises an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin, such as of from 2.0 µg to 200 mg ghrelin, such as from 5.0 µg to 100 mg ghrelin, such as from 10 µg to 50 mg ghrelin, such as from 10 µg to 5 mg ghrelin, such as from 10 µg to 1.0 mg ghrelin.

The medical packaging may be in any suitable form for parenteral, in particular subcutaneous administration. In a preferred embodiment the packaging is in the form of a cartridge, such as a cartridge for an injection pen, the injection pen being such as an injection pen known from insulin treatment.

When the medical packaging comprises more than one dosage unit, it is preferred that the medical packaging is provided with a mechanism to adjust each administration to one dosage unit only.

Preferably, a kit contains instructions indicating the use of the dosage form to achieve a desirable affect and the amount of dosage form to be taken over a specified time period. Accordingly, in one embodiment the medical packaging comprises instructions for administering the pharmaceutical composition. In particular said instructions may include instructions referring to administration of said pharmaceutical composition either during a meal, or preferably at the most 45 minutes prior to a meal, such as at the most 30 minutes prior to a meal, such as at the most 25 minutes prior to a meal, such as at the most 20 minutes prior to a meal, such as at the most 15 minutes prior to a meal, such as at the most 10 minutes prior to a meal, such as at the most 5 minutes prior to a meal.

Examples

Example 1

Competition binding assays

Transfected COS-7 cells were transferred to culture plates one day after transfection at a density of 1 x 10^5 cells per well aiming at 5 - 8 % binding of the radioactive li-

gand. Two days after transfection competition binding experiments were performed for 3 hours at 4EC using 25 pM of ^{125}I-ghrelin (Amersham, Little Chalfont, UK). Binding assays were performed in 0.5 ml of a 50 mM Hepes buffer, pH 7.4, supplemented with 1 mM $CaCl_2$, 5 mM $MgCl_2$, and 0.1 % (w/v) bovine serum albumin, 40 :g/ml bacitracin. Non-specific binding was determined as the binding in the presence of 1 :M of unlabeled ghrelin. Cells were washed twice in 0.5 ml of ice-cold buffer and 0.5-1 ml of lysis buffer (8 M Urea, 2 % NP40 in 3 M acetic acid) was added and the bound radioactivity was counted. Determinations were made in duplicate. Initial experiments showed that steady state binding was reached with the radioactive ligand under these conditions.

Example 2

Receptor activation assays

One simple measure of the ability of a ghrelin like compound to activate the ghrelin receptor is to measure its EC50, i.e. the dose at which the compound is able to activates the signalling of the receptor to half of the maximal effect of the compound. The ghrelin receptor can either be expressed endogenously on primary cells cultures, for example pituitary cells, or heterologously expressed on cells transfected with the ghrelin receptor. Whole cell assays or assays using membranes prepared form either of these cell types can be used depending on the type of assay.

As the ghrelin receptor is generally believed to be primarily coupled to the Gq signalling pathway, any suitable assay which monitor activity in the Gq/G11 signalling pathway can be used, for example:

1) an assay measuring the activation of Gq / G11 performed for example by measurement of GTPgS binding combined with, e.g., anti-Gα–q or -11 antibody precipitation in order to increase the signal to noise ratio. This assay may also detect coupling to other G-proteins than Gq/11.

2) An assay which measure the activity of phopholipase C (PLC) one of the first down-stream effector molecules in the pathway, for example by measuring the accumulation of inositol phosphate which is one of the products of PLC.

3) More down stream in the signalling cascade is the mobilization of calcium from the intracellular stores

4) Further more down stream signalling molecules such as the activity of different kinds of MAP kinases (p38, jun, ect.), NF-κ-B translocation and CRE driven gene transcription may also be measured.

5) Alternatively binding of fluorescently tagged arrestin to the activated ghrelin receptor may also be used.

Example 3

Synthetic production of ghrelin-like compound

Amino acid derivatives and synthesis reagents, were obtained from commercial sources. Peptide chain extension was performed by mainly using Applied Biosystem 433A synthesizer produced by Perkin Elmer, and a protected peptide derivative-resin was constructed by the Boc or Fmoc method. The protected peptide resin obtained by the Boc method was deprotected with anhydrous hydrogen fluoride (HF) in the presence of p-cresol thereby releasing the peptide, which was then purified. The protected peptide resin obtained by the Fmoc method was deprotected with trifluoroacetic acid (TFA) or dilute TFA containing various scavengers, and the released peptide was purified. Purification was performed in reversed phase HPLC on a C4 or C18 column. The purity of the purified product was confirmed by reverse phase HPLC, and its structure was confirmed by amino acid composition analysis and mass spectrometry.

The peptide of the present invention is produced by a conventional peptide synthesis method. Specifically, synthesis of acylated or alkylated peptides is exemplified below. Further, human-derived ghrelin (which may be abbreviated hereinafter to hGhrelin) or rat-derived ghrelin (which may be abbreviated hereinafter to rGhrelin) was reacted with trypsin or chymotrypsin or both the enzymes successively to give the following ghrelin fragments: 19. Ghrelin (16-28), 20. hGhrelin (1-15), 21. rGhrelin (1-15), 23. hGhrelin (1-11), 24. rGhrelin (1-11), 25. Ghrelin (1-10), 26. Ghrelin (1-9),

27. Ghrelin (1-8), and 30. Ghrelin (1-4). Then, these fragments were isolated by analytical HPLC and measured for their activity. 41. [N-Acetyl]-Ghrelin (1-10) was prepared in a usual manner by treating Ghrelin (1-10) with N-acetylsuccinimide. Human and rat ghrelin may also be made by use of a natural material.

Abbreviations

HMP resin; 4-hydroxymethyl-phenoxymethyl resin
Fmoc amide resin; 4-(2', 4'-dimethoxyphenyl-Fmoc-aminomethyl) phenoxyacetamido-ethyl resin
PAM resin; phenylacetoamidomethyl resin
HBTU; 2-(IH-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
TBTU; 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
HOBt; 1-hydroxybenzotriazole
DCC; dicyclohexylcarbodiimide
DIPCI; diisopropylcarbodiimide
TFA; trifluoroacetic acid
DIPEA; diisopropylethylamine
TIPS; triisopropylsilane
Fmoc; fluorenylmethoxycarbonyl
Boc; t-butyloxycarbonyl
Trt; trityl
Bu; t-butyl
Pmc; 2,2,5,7,8-pentamethylchroman-6-sulfonyl
Prl; propionyl
PhPrl; phenylpropionyl
Bzl; benzyl
Bom; benzyloxymethyl
Tos; toluenesulfonyl
Cl-Z; 2-chloro-benzyloxycarbonyl
Pis; 2-phenylisopropyl
Mtt; 4-methyltrityl
DMF; N,N-dimethylformamide
NMP; N-methylpyrrolidone
DMAP; 4-dimethylaminopyridine

HOSu; N-hydroxysucciniimide
Adod; 2-aminododecanoic acid
Aib; 2-aminoisobutylic acid
Ape; 5-aminopentanoic acid
Cha; cyclohexylalanine
Dap; 2, 3-diaminopropionic acid
Nal; naphtylalanine
Nle; norleucine

Protecting amino acids used in synthesis

Fmoc method:

Boc-Gly, Fmoc-Gly, Fmoc-Ser (Bu), Fmoc-Ser (Trt), Fmoc-Glu (OBu), Fmoc-His (Boc), Fmoc-Gln (Trt), Fmoc-Arg (Pmc), Fmoc-Lys (Boc), Fmoc-Pro, Fmoc-Leu, Fmoc-Ala, Fmoc-Val, Fmoc-Phe, Fmoc-Phe, Fmoc-Ser (n-C8H17), Fmoc-Ser (n-C8H17), Fmoc-Cys (n-C8H17), Fmoc-Asp (OPis), Fmoc-Ser (Bzl), Fmoc-Cys (Trt), Fmoc-Dap (Octanoyl), Fmoc-2-Nal, Fmoc-2-Nal, Fmoc-Nle, Fmoc-Lys (Mtt), Fmoc-Aib-OH, Fmoc-Asp (O-C7H15)

Boc method:

Boc-Gly, Boc-Ser (Bzl), Boc-Ser (Ac), Boc-Ser (Prl), Boc-Glu (OBzl), Boc-His (Bom), Boc-Gln, Boc-Arg (Tos), Boc-Lys (Cl-Z), Boc-Pro, Boc-Leu, Boc-Ala, Boc-Val, Boc-Phe, Boc-Cys (n-C8H17), Boc-Ape Boc-Ser (n-C8H17)

Units used

(a) Analytical HPLC system

Unit: Shimadzu LC-10A System
Column: YMC PROTEIN-RP (4.6 mm phi x150 mm)
Column temperature: 40 DEG C
Eluent: A linear gradient of from 0 to 50 % acetonitrile for 20 minutes in 0.1% trifluoroacetic acid
Flow rate: 1 mL/min.

Detection: UV (210 nm)
Injection volume: 10 to 100 mu l

Preparative HPLC system

Unit: Waters 600 Multisolvent Delivery System
Columns:

YMC-Pack-ODS-A (5 mu m, 20 mmx250 mm)
YMC-Pack-PROTEIN-RP (5 mu m, C4, 10 mmx250 mm)
YMC-Pack PROTEIN-RP (5 mu m, C4, 20 mmx250 mm)
YMC PROTEIN-RP (4.6 mm phi x150 mm)
Eluent: A suitable linear gradient of acetonitrile concentration in 0.1 % trifluoroacetic acid
Flow rate: 10 mL/min. (for the column of an inner diameter of 20 mm), 3 mL/min. (for the column of an inner diameter of 10 mm), 1 mL/min. (for the column of an-inner diameter of 4.6 mm) Detection: 210 nm, 260 nm
Injection: 10 to 2000 mu l (2000 mu l or more was injected via a pump)

(c) Mass spectrometer

Unit: Finigan MAT TSQ700
Ion source: ESI
Detection ion mode: Positive
Spray voltage: 4.5 kV
Capillary temperature: 250 DEG C
Mobile phase: A mixture of 0.2% acetic acid and methanol (1 : 1)
Flow rate: 0.2 mL/min.
Scan range: m/z 300 to 1,500

(d) Analysis of amino acid sequence

Unit: Applied Biosystem 477A, 492 model sequencer manufactured by Perkin Elmer

(e) Analysis of amino acid composition

Unit: L-8500 model amino acid analyzer manufactured by Hitachi,Co., Ltd.

Sample: Unless otherwise specified, the sample was hydrolyzed with 6 M HCl at 110 DEG C for 24 hours in a sealed tube.

Example of synthesis of a derivative having acyl serine (Fmoc method, carboxyl-terminal amide derivatives)

hGhrelin: GSS(CO-C7H15)FLSPEHQRVQQRKESKKPPAKLQPR

Fmoc-Arg(Pmc)-HMP-resin (403 mg, 0.25 mmol, ABI Co., Ltd) was treated with 20% piperazine for 20 minutes and subjected repeatedly to introduction of Fmoc-amino acid by HBTU/HOBt and elimination of Fmoc by piperazine sequentially to construct Fmoc-Ser(Bu)-Ser(Trt)-Phe-Leu-Ser(tBu)-Pro-Glu(OBu)-His(B oc)-Gln(Trt)-Arg(Pmc)-Val-Gln(Trt)-Gln(Trt)-Arg(Pmc)-Lys(Bo c)-Glu(OBu)-Ser(Bu)-Lys(Boc)-Lys(Boc)-Pro-Pro-Ala-Lys(Boc)-Leu-Gln(Trt)-Pro-Arg(Pmc)-resin. After Boc-Gly was finally introduced by DCC/HOBt, the resulting protected peptide resin (1.3 g) was treated with 1 % TFA-5 % TIPS-methylene chloride solution (15 mL) for 30 minutes. The peptide resin was filtrated, washed several times with methylene chloride (30 mL), and washed with 5 % DIEA (10 mL) and then with methylene chloride (30 mL). The resulting de-Trt peptide resin (about 1.3 g) was swollen with NMP (10 mL), and octanoic acid (144.2 mg, 1.0 mmol) and DIPCI (126.2 mg, 1.0 mmol) were added thereto in the presence of DMAP (61.1 mg, 0.5 mmol) and allowed to react for 8 hours. The resin was recovered by filtration and washed with NMP and then with methylene chloride, followed by drying under vacuum to give about 1.2 g protected peptide resin wherein the side chain of 3rd serine was octanoylated. To this product was added a de-protecting reagent (10 mL) consisting of 88 % TFA-5 % phenol-2% TIPS-5 % H2O, and the mixture was stirred at room temperature for 2 hours. The resin was removed by filtration, and the filtrate was concentrated followed by adding ether to the resulting residues to form precipitates. The precipitates were recovered by filtration and dried to give about 550 mg crude peptide. 200 mg of this product was dissolved in 10 mL water and applied to YMC-Pack PROTEIN-RP (C4, 20 mmx250 mm) and eluted with a linear gradient (flow rate: 10 mL/min.) for 60 min-

utes of from 0 to 54 % acetonitrile in 0.1% trifluoroacetic acid. The desired fractions were collected and lyophilized to give about 120 mg of the desired product.

Example of synthesis of a derivative having acyl serine (Fmoc method, carboxyl-terminal amide compoundsi)

Ghrelin (1-9)-NH2; GSS(CO-C7H15)FLSPEH-NH2

Fmoc-amide-resin (403 mg, 0.25 mmol, ABI Co.,Ltd) was treated with 20% piperazine for 20 minutes and subjected repeatedly to introduction of Fmoc-amino acid by HBTU/HOBt and elimination of Fmoc by piperazine sequentially to construct Fmoc-Ser(Bu)-Ser(Trt)-Phe-Leu-Ser(Bu)-Pro-Glu(OBu)-His(Bo c)-resin. After Boc-Gly was finally introduced by DCC/HOBt, the resulting protected peptide resin (about 550 mg) was treated with 1 % TFA-5 % TIPS-methylene chloride solution (10 mL) for 30 minutes. The peptide resin was recovered by filtration, washed several times with methylene chloride (30 mL), and washed with 5 % DIEA (10 mL) and then with methylene chloride (30 mL). The resulting de-Trt peptide resin (about 750 mg) was swollen with NMP (10 mL), and octanoic acid (144.2 mg, 1.0 mmol) and DIPCI (126.2 mg, 1 mmol) were added thereto in the presence of DMAP (61.1 mg, 0.5 mmol) and allowed to react for 4 hours. The resin was recovered by filtration and washed with NMP and then with methylene chloride, followed by drying under vacuum to give about 800 mg protected peptide resin wherein the side chain of 3rd serine was octanoylated. TFA (10 mL) was added to this product and stirred at room temperature for 30 minutes. The resin was removed by filtration, and the filtrate was concentrated followed by adding ether to the resulting residues to form precipitates. The precipitates were recovered by filtration and dried to give about 250 mg crude peptide. About 200 mg of this product was dissolved in 10 mL of 30 % aqueous acetic acid and applied to YMC-Pack PROTEIN-RP (C4, 20 mmx250 mm) and eluted with a linear gradient (flow rate: 10 mL/min.) for 60 minutes of from 0 to 54 % acetonitrile in 0.1% trifluoroacetic acid. The desired fractions were collected and lyophilized to give about 150 mg of the desired product.

Example of synthesis of a derivative having acyl serine (Boc method)

[Ser3(Propionyl)]-rGhrelin (1-28);

GSS(CO-CH2CH3)FLSPEHQKAQQRKESKKPPAKLQPR

Protected rat ghrelin resin (4-28) was constructed from Boc-Arg (Tos)-Pam resin (0.75 g, 0.5 mmol) by Boc chemistry, and Boc-Ser (CO-CH2CH3)-OH, Boc-Ser (Bzl)-OH and Boc-Gly-OH were condensed with a half (1.4 g) of the resin. The resulting resin, 1.5 g, was treated with a mixture of HF and p-cresol (8.5 mL : 1.5 mL) at 0 DEG C for 1 hour, and the HF was evaporated. Ether was added to the residues, whereby 671 mg crude peptide was obtained. This sample was dissolved in 50% acetic acid (AcOH) and applied to a preparative column YMC-Pack-ODS-A (5 mu m, 20 mmx250 mm) and eluted at a rate of 10 mL/min. by a gradient of from 0 to 95 % acetonitrile concentration in 0.1% TFA solution for 75 minutes. Those fractions containing the desired product were lyophilized to give 135.8 mg crude peptide. A part (0.5 mg) of this product was applied to YMC-A-302 column (C18, 4.6 mmx150 mm) and eluted at a flow rate of 1 mL/min. by a gradient of from 15 to 19% concentration acetonitrile. This purification procedure was repeated and the desired fractions were combined to give 0.41 mg of the desired product.

Other compounds according to the invention may be produced likewise.

Example 4

A randomised, single centre, four-period cross-over trial to investigate the absolute bioavailability of iv administered Ghrelin and sc administered Ghrelin at three different single doses in healthy subjects.

Objectives:

Primary: To investigate the absolute bioavailability of three different doses of Ghrelin administered as single iv and sc doses.

Secondary: 1) To investigate the dose linearity (dose proportionality) of the ascending doses. 2) To investigate and compare the pharmacodynamic profiles between the treatments. 3) To assess the safety and local tolerability.

Trial Design:

A randomised, single centre, unbalanced block design, four-period cross-over trial to investigate the absolute bioavailability between iv administered Ghrelin and sc administered Ghrelin at three different single doses in healthy subjects. Three doses will be used for each way of administration: low, medium and high. To reduce the number of dosings to each individual and hence reduce the length of the trial each subject will only receive four doses of the total of six doses, ie. two dose levels administered as iv and sc, respectively. The unbalanced block design will ensure that all three-dose levels will be covered in this way although not all subjects will receive all dose levels. A sufficient washout period will be placed between the individual dosing periods.

Endpoints:

Pharmacokinetics of Ghrelin:

AUC_{0-t}, AUC, C_{max}, t_{max}, $t_{½}$, Cl/f, Vz/f, Cl, Vz, , $t_{½}$, MRT

Pharmacodynamics:

GH: AUC, Cmax and tmax

Cardiac output, assessment of hunger, food/energy intake, degree of pleasure related to food intake, body mass, energy expenditure, DEXA.

Safety:

Safety and local tolerability will be assessed throughout the study by clinical evaluations (physical examination and vital signs), electrocardiography and laboratory tests (hematology and clinical chemistry).

Trial population and power calculation:

Healthy male subjects, aged 18-45 years with a body mass index (BMI) of 19-26 kg/m^2 (both inclusive).

The primary objective of this study is to investigate the absolute bioavailability of ghrelin administered as iv and sc. An unbalanced block design will be applied to reduce the trial period time and reduce the number of dosings per subject. The number of subjects needed to perform a statistical analysis of absolute bioavailability per dose levels as well as an analysis of dose linearity between doses will be calculated based on existing literature data.

Trial products:

Ghelin for iv and sc administration.

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SEQUENCE LISTING

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<120> Use of Ghrelin-like compound

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Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
20 25

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<223> Amino acid in position 3 is modified with a fatty acid

<400> 3

Gly Ser Ser Phe Leu Ser Pro Glu His Gln Lys Ala Gln Gln Arg Lys
1 5 10 15

Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg
20 25

Claims

1. Use of a ghrelin-like compound or a pharmaceutically acceptable salt thereof for the preparation of a medicament for one or more of

prophylaxis or treatment of cachexia, and/or

prophylaxis or treatment of lipodystrophy, and/or

stimulation of appetite, and/or

stimulation of food intake, and/or

stimulation of weight gain, and/or

increasing body fat mass, and/or

in an individual by administering a subcutaneous dosage of said medicament to the individual,

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of X^1 and X^3 optionally may be modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

2. Use according to claim 1, wherein m is an integer in the range of from 1-9, such as of from 1-8, such as of from 1-7, such as of from 1-6, such as of from 1-5, such as of from 1-4, such as of from 1-3, such as of from 1-2, such as 2,

3. Use according to any of the preceding claims, wherein X^2 is selected from the group of modified Ser, modified Cys and modified Lys, such as wherein X^2 is modified Ser.

4. Use according to any of the preceding claims, wherein the ghrelin-like compound is selected from a compound of

formula II $Z^1 - Gly- (X^1)_{m-1} - (X^2) - (X^3)_n- Z^2$,

formula III $Z^1 - Gly- Ser - (X^2) - (X^3)_n- Z^2$, and

formula IV $Z^1 - Gly - (X^2) - (X^3)_n- Z^2$.

5. Use according to claim 4, wherein the ghrelin-like compound is having formula III.

6. Use according to any of the preceding claims, wherein $(X^3)_n$ comprises a sequence selected from one or more of the sequences shown below:

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

7. Use according to any of the preceding claims, wherein n is an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24.

8. Use according to any of the preceding claims, wherein $(X^3)_n$ is selected from one or more of the sequences shown below:

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln

Phe Leu Ser Pro Glu His Gln Arg Val

Phe Leu Ser Pro Glu His Gln Arg

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

9. Use according to any of the preceding claims, wherein the acyl group is selected from a C1-C35 acyl group, such as a C1 – C20 acyl group, such as a C1 – C15 acyl group, such as a C6 – C15 acyl group, such as a C6 – C12 acyl group, such as a C8 – C12 acyl group.

10. Use according to any of the preceding claims, wherein the acyl group is selected from the group of C7 acyl group, C8 acyl group, C9 acyl group, C10 acyl group, C11 acyl group, and C12 acyl group.

11. Use according to any of the preceding claims, wherein the acyl group is selected from the group of C8 acyl group, and C10 acyl group.

12. Use according to any of the preceding claims, wherein the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

13. Use according to any of the preceding claims, wherein the medicament is in a formulation for subcutaneous administration.

14. Use according to claim 13, wherein the formulation comprises the ghrelin-like compound or a pharmaceutically acceptable salt thereof.

15. Use according to any of the preceding claims 13 or 14, wherein the formulation comprises the ghrelin-like compound or a salt thereof as a lyophilisate and the formulation further comprises a solvent, said lyophilisate and said solvent being in separate compartements until administration.

16. Use according to any of the preceding claims 13 or 14, wherein the formulation is a solution of the ghrelin-like compound or a salt thereof.

17. Use according to claim 15 or 16, wherein the solvent is saline.

18. Use according to any of the preceding claims, wherein the medicament is administered before a meal or during the intake of a meal.

19. Use according to any of the preceding claims, wherein the medicament is administered in a concentration equivalent to from 10 ng to 10 mg ghrelin per kg body weight.

20. Use according to claim 19, wherein the medicament is administered in a concentration equivalent to from 0.1 µg to 1 mg ghrelin per kg bodyweight, such as from 0.5 µg to 0.5 mg ghrelin per kg bodyweight, such as from 1.0 µg to 0.1 mg ghrelin per kg bodyweight, such as from 1.0 µg to 50 µg ghrelin per kg bodyweight, such as from 1.0 µg to 10 µg ghrelin per kg bodyweight.

21. Use according to any of the preceding claims, wherein the medicament is administered as a bolus injection prior to or during the intake of a meal, said bolus injection comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin.

22. Use according to claim 21, wherein the medicament is administered as a bolus injection prior to or during the intake of a meal, said bolus injection comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 2.0 µg to 200 mg ghrelin, such as from 5.0 µg to 100 mg ghrelin, such as from 10 µg to 50 mg ghrelin, such as from 10 µg to 5 mg ghrelin, such as from 10 µg to 1.0 mg ghrelin.

23. Use according to any of the preceding claims, wherein the medicament is administered from one to three times daily, each administration being prior to or during a meal, preferably less than 180 minutes prior to a meal, such as less than 90 minutes prior to a meal, for example less than 45 minutes prior to a meal, such as less than 30 minutes prior to a meal, for example less than 25

minutes prior to a meal, such as less than 20 minutes prior to a meal, for example less than 15 minutes prior to a meal, such as about 10 minutes prior to a meal, for example about 5 minutes prior to a meal, such as immediately prior to a meal, or during a meal, such as less than 90 minutes after commencing a meal, for example less than 45 minutes after commencing a meal, such as less than 30 minutes after commencing a meal, for example less than 25 minutes after commencing a meal, such as less than 20 minutes after commencing a meal, for example less than 15 minutes after commencing a meal, such as less than 10 minutes after commencing a meal, for example less than 5 minutes after commencing a meal.

24. Use according to claim 23, wherein the medicament is administered from one to three times daily, preferably once prior to or during breakfast and/or once prior to or during lunch and/or once prior to or during dinner.

25. Use of a ghrelin-like compound or a pharmaceutically acceptable salt thereof for the preparation of a medicament for one or more of

prophylaxis or treatment of cachexia, and/or

prophylaxis or treatment of lipodystrophy, and/or

stimulation of appetite, and/or

stimulation of food intake, and/or

stimulation of weight gain, and/or

increasing body fat mass, and/or

in an individual by administering a dosage of said medicament to the individual prior to or during a meal, said dosage comprising an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin,

wherein the ghrelin-like compound comprises a structure defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic occurring amino acids, said amino acid being modified with a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

Z^2 is an optionally present protecting group,

m is an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

26. Use according to claim 25, wherein the medicament is administered as a bolus injection or by fast running infusion.

27. Use according to any of claims 25 and 26, having one or more of the features of the claims 1-24.

28. A ghrelin-like compound wherein the ghrelin-like compound is defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic amino acids, said amino acid being modified with an acyl group, wherein the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids.

Z^2 is an optionally present protecting group,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

m is 0 or an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

29. The compound according to claim 28, wherein m is an integer in the range of from 1-9, such as of from 1-8, such as of from 1-7, such as of from 1-6, such as of from 1-5, such as of from 1-4, such as of from 1-3, such as of from 1-2, such as 2.

30. The compound according to any of claims 28 and 29, wherein X^2 is selected from the group of modified Ser, modified Cys and modified Lys, such as wherein X^2 is modified Ser.

31. The compound according to any of the claims 28 to 30, wherein the ghrelin-like compound is selected from a compound of

formula II $Z^1 - Gly- (X^1)_{m-1} - (X^2) - (X^3)_n- Z^2$,

formula III Z^1 – Gly- Ser – (X^2) – $(X^3)_n$- Z^2, and

formula IV Z^1 – Gly – (X^2) – $(X^3)_n$- Z^2.

32. The compound according to claim 31, wherein the ghrelin-like compound is having formula III.

33. The compound according to any of claims 28 to 32, wherein n is an integer in the range of from 1-25, such as of from 1-24, such as from 1-15, such as of from 1-10, such as of from 10-25, such as of from 10-24, such as of from 15-25, such as of from 15-24.

34. The compound according to any of claims 28 to 33, wherein $(X^3)_n$ comprises a sequence selected from one or more of the sequences shown below:

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

35. The compound according to any of claims 28-34, wherein $(X^3)_n$ is selected from one or more of the sequences shown below:

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro Arg

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys Leu

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro Ala

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys Pro

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu Ser

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys Glu

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg Lys

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln Arg

Phe Leu Ser Pro Glu His Gln Arg Val Gln Gln

Phe Leu Ser Pro Glu His Gln Arg Val Gln

Phe Leu Ser Pro Glu His Gln Arg Val

Phe Leu Ser Pro Glu His Gln Arg

Phe Leu Ser Pro Glu His Gln

Phe Leu Ser Pro Glu His

Phe Leu Ser Pro Glu

Phe Leu Ser Pro

Phe Leu Ser

Phe Leu

Phe

36. A pharmaceutical composition comprising the compound as defined in any of the claims 28 to 35 or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients.

37. The pharmaceutical composition according to claim 36, wherein said composition further comprising transport molecules, such as liposomes, micelles, iscoms, and/or microspheres.

38. A pharmaceutical composition comprising the compound as defined in any of the claims 1 to 35, or a pharmaceutically acceptable salt thereof, and pharmaceutically acceptable carriers, vehicles and/or excipients said composition further comprising transport molecules, such as liposomes, micelles, iscoms, and/or microspheres.

39. A medical packaging comprising one or more dosage units of a pharmaceutical composition comprising a compound as defined in any of claims 1-35 or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients.

40. The medical packaging according to claim 39, said packaging comprising from one to three dosage units, such as three dosage units.

41. A medical packaging comprising a pharmaceutical composition comprising a compound as defined in any of claims 1 to 35 or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients, said packaging having from one to three dosage units.

42. The medical packaging according to claim 41, having three dosage units.

43. A medical packaging comprising a pharmaceutical composition comprising a compound as defined in any of claims 1 to 35 or a pharmaceutically acceptable salt thereof and pharmaceutically acceptable carriers, vehicles and/or excipients, said packaging having from 7 to 21 dosage units.

44. The medical packaging according to claim 43, having 7, 14, or 21 dosage units.

45. The medical packaging according to any of the claims 39 to 44, wherein said dosage unit comprises an amount of the ghrelin-like compound or a salt thereof equivalent to from 0.3 µg to 600 mg ghrelin, such as of from 2.0 µg to 200 mg ghrelin, such as from 5.0 µg to 100 mg ghrelin, such as from 10 µg to 50 mg ghrelin, such as from 10 µg to 5 mg ghrelin, such as from 10 µg to 1.0 mg ghrelin.

46. The medical packaging according to any of the claims 39 to 45, comprising instructions for administering the pharmaceutical composition.

47. The medical packaging according to claim 46, wherein said instructions include instructions referring to administration of said pharmaceutical composition during a meal or at the most 90 minutes prior to a meal, such as at the most 45 minutes

prior to a meal, such as at most 30 minutes prior to a meal, such as at the most 25 minutes prior to a meal, such as at the most 20 minutes prior to a meal, such as at the most 15 minutes prior to a meal, such as at the most 10 minutes prior to a meal, such as at the most 5 minutes of a meal, such as immediately prior to a meal.

48. The medical packaging according to any of claims 39-47, wherein the packaging is in the form of a cartridge, such as a cartridge for an injection pen.

49. Use of a ghrelin-like compound or a pharmaceutically acceptable salt thereof, for the preparation of a medicament for the treatment in an individual of a clinical indication responsive to treatment with the ghrelin-like compound,

wherein the ghrelin-like compound is defined by formula I

$Z^1 - (X^1)_m - (X^2) - (X^3)_n - Z^2$, wherein

Z^1 is an optionally present protecting group

each X^1 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

X^2 is any amino acid selected from naturally occurring and synthetic amino acids, said amino acid being modified with an acylgroup, wherein the acyl group is selected from the group of C7 acyl group, C9 acyl group, and C11 acyl group, such as from the group of C9 acyl group and C11 acyl group.

each X^3 is independently selected from an amino acid, wherein said amino acid is selected from naturally occurring and synthetic amino acids,

Z^2 is an optionally present protecting group,

wherein one or more of X^1 and X^3 optionally may be modified by a bulky hydrophobic group, preferably an acyl group, or a fatty acid,

m is 0 or an integer in the range of from 1-10

n is 0 or an integer in the range of from 1-35.

50. The use according to claim 49, wherein the compound has one or more of the features defined in claims 1-35.